Federal Institute For Risk Assessment

International collaborative study for the Determination of pyrrolizidine alkaloids in honey and herbal tea by SPE-LC-MS/MS

(BfR-PA-Honey-1.0_2013)
(BfR-PA-Tea-1.0_2013)

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Content

Summary

The Federal Institute for Risk Assessment (BfR) organised a collaborative study with 20 participating laboratories for the determination of 17 pyrrolizidine alkaloids (PA) in honey and tea. The study was conducted according to the harmonised protocol of ISO/IUPAC/AOAC and included the analyses of six samples and one recovery sample per matrix (honey, tea). Meanwhile, the clean-up method for the determination of PA in black and green tea was modified and the results for the black tea sample were, therefore, excluded from statistical evaluation. For the updated method protocol that now also includes a description for homogenisation of laboratory samples, please refer to
www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-in-plant-material.pdf
(English version)
or
www.bfr.bund.de/cm/343/bestimmung-von-pyrrolizidinalkaloiden.pdf
(German version).

All participants submitted their results in time. The evaluation of the data sets was carried out according to ISO 5725.

Excellent repeatability and reproducibility precision were achieved for the five naturally contaminated honey samples together with excellent recovery rates for the spiked recovery honey sample. The HorRat values ranged between 0.4 and 1.0 with recovery rates between 75 % and 103 % for the provided test sample. This indicates that the method is applicable for the determination of PA in honey.

The evaluation of the tea samples showed sufficient reproducibility precision for the naturally contaminated samples as well as acceptable recovery rates between 76 % and 125 % for the spiked recovery sample. The results for teas demonstrated that the sample pretreatment, especially the degree of homogenisation, has a critical influence on repeatability and reproducibility of the method. Furthermore, the evaluation suggested that the chromatographic separation of isomeric compounds that may differ between laboratories has a decisive influence to the quantification of PA in tea.

Nevertheless, the study demonstrated due to the excellent HorRat values obtained for the recovery sample, that the method is applicable for the determination of PA in tea.

1 Introduction

1.1 Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PA) are secondary plant compounds produced by a large number of plant species mainly of the families *Asteraceae*, *Fabaceae* and *Boraginaceae*. The occurrence of PA in plants varies widely, depending on the plant species and the part of the plants. Due to their potentially harmful effects, pyrrolizidine alkaloids in particular are undesirable in food and feed, as they can lead to acute liver damage when consumed in high doses. In animal studies, some PA were classified as genotoxic carcinogens [1-4]. PA are esters from a 1-hydroxymethylpyrrolizidine (necine base) and aliphatic monocarboxcylic or dicarboxylic acids. A structural distinction of the necine base is made between retronecine-type, heliotridine-type, the otonecine-type and the platynecine-type (Figure 1).

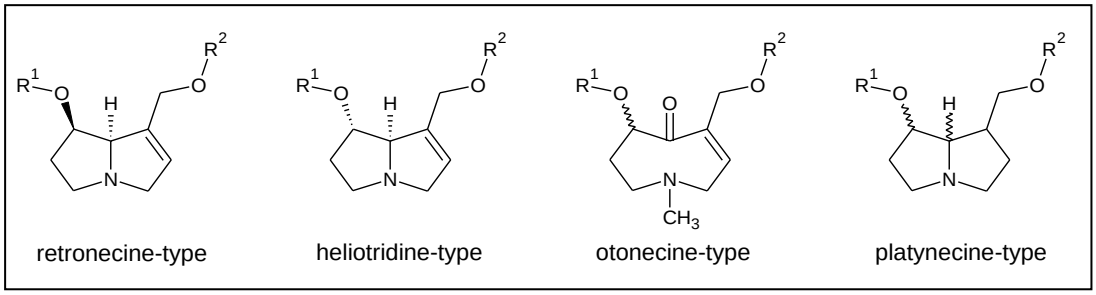

Figure 1: Necine bases

Depending on the esterification of one or both hydroxyl groups, pyrrolizidine alkaloids can occur as monoesters or diesters. Condensation of two necic acids results in a cyclic diester. In plants, they occur mainly oxidized at the nitrogen position (PA-*N*-oxides) (Figure 2) [5].

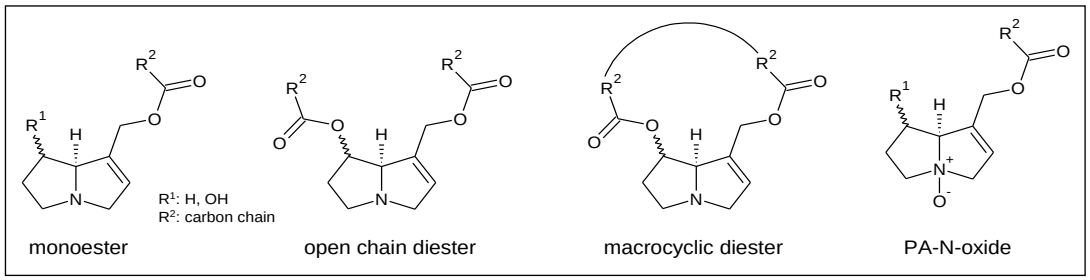

Figure 2: Different types of PA esterification including an N-oxide example

1.2 Aim of the collaborative study for the determination of PA in honey and tea

Aim of the collaborative study was the validation of the analytical methods (BfR-PA-Honey-1.0_2013; BfR-PA-Tea-1.0_2013) for the quantitative determination of 17 commercially available pyrrolizidine alkaloids (PA) in honey and tea by LC-MS/MS after aqueous extraction and solid phase extraction.

The study included the quantitative determination of the following PA:
echimidine (Em), heliotrine (Hn), heliotrine-N-oxide (HnN), intermedine (Im), lasiocarpine (Lc), lasiocarpine-N-oxide (LcN), lycopsamine (La), monocrotaline (Mc), monocrotaline-N-oxide (McN), retrorsine (Re), retrorsine-N-oxide (ReN), seneciphylline (Sp), seneciphylline-N-oxide (SpN), senecionine (Sc), senecionine-N-oxide (ScN), senkirkine (Sk) and trichodesmine (Td).

2 Strategy and organisation of the collaborative study

2.1 Motivation

The BfR initiated a research project for the determination of PA in food and feed. As a part of this research project 120 honey samples from the international retail market were analysed by a modified method according to Betteridge et al. [7].The analytical method for honey is based on acidic extraction, clean-up and enrichment by means of solid phase extraction (SPE) using a strong cation exchange phase (SCX) followed by liquid chromatographic separation and mass spectrometric detection (LC-MS/MS). Furthermore, in the frame of this project 274 different herbal tea and tea samples purchased from the German retail market were analysed. For this purpose an analytical method for the determination of PA in herbal tea and tea was developed and in-house validated. The method comprises an acidic extraction, clean-up and enrichment using a C18-SPE and detection by LC-MS/MS. Thereby, total PA contents (sum of 17 individual PA) up to 5647 µg/kg were determined in the dry products of herbal tea and tea samples [8]. Due to the obtained data for tea and herbal tea and the resulting demand on the availability of validated methods the BfR decided to conduct a collaborative study to validate methods for both: tea and honey. A prevalidation round was conducted in preparation of the validation.

2.2 Prevalidation round

A total of 24 laboratories participated in the prevalidation round, whereby results of 18 laboratories were statistically evaluated. The aim was to familiarise the participants with both methods:

- Determination of PA in honey (BfR-PA-Honey-1.0_2013)
- Determination of PA in plant material (BfR-PA-Tea-1.0_2013)

Furthermore, first information on performance and reproducibility of the suggested methods should be derived. For these purposes the preliminary study involved the quantitative determination of PA in two honey samples (1x polyfloral honey, 1x honeydew honey) and two herbal tea samples (melissa tea, chamomile tea) using a matrix matched calibration. The study was also focussed on assessment of repeatability of duplicate measurement of one sample and potential response drift of the LC-MS/MS signal during one measured sequence. Additionally, the linearity of the MS detectors was evaluated by analysing several calibration levels which cover a wide concentration range (herbal tea: 2 µg/kg – 600 µg/kg; honey: 0.1 µg/kg – 12.5 µg/kg).

The results indicated a sufficient reproducibility for both methods for the provided honey and tea samples, both methods were considered to be applicable for the main validation study. The different calibration curves analysed by the participants demonstrated that a concentration range from 10 µg/kg to 300 µg/kg for tea and from 0.1 µg/kg to 10 µg/kg for honey should be adequate.

2.3 Time table

- Invitation to participate in the prevalidation round: February 2013
  - Dispatch of samples: March 2013
  - Submission of results: June 2013
  - Dispatch of Draft report: July 2013
- Invitation to participate in the main study at the end of July 2013
  - Dispatch of samples: beginning of September 2013
  - Submission of results: end of November 2013
  - Dispatch of Draft report: 2014

3 Main study

3.1 Test Materials and Methods

3.1.1 Sample preparation (honey)

Samples were obtained by mixing different honeys purchased from the German retail market. Additionally, four test samples were blended with a highly contaminated raw honey. All test samples were homogenized for three hours at a temperature of 40 °C using a kitchen machine (Kenwood KM070) and subsamples of 10 g of honey were filled into plastic centrifuge tubes. All honey samples were stored at 4 °C until shipment. Further information and the labelling of the honey samples are given in Table 1.

Table 1: Description of the honey samples

Sample name	Description	Fortified with raw honey
HO_01	blossom honey	no
HO_02	summer blossom honey	yes
HO_03	blossom honey	no
HO_04	blossom honey	yes
HO_05	orange blossom honey	yes
HO_06	summer blossom honey	yes
HO_Rec	cornflower honey*	---
* purchased directly from beekeeper		

3.1.2 Sample preparation (tea)

All tea samples were purchased from retail markets in the Berlin area. 500 g of each sample were milled (particle size ≤ 500 µm) and homogenised in a glass container for three hours using an overhead shaker. Subsequently, 2 g of the samples were filled into plastic centrifuge tubes, sealed to be airtight and stored at room temperature until shipment. Further information and the labelling of the tea samples are given in table 2.

Table 2: Description of the tea samples

Sample name	Description
PM_01	melissa tea
PM_02	chamomile tea
PM_03	mixed herbal tea
PM_04	rooibos tea flavoured with vanilla
PM_05	melissa tea
PM_06	black tea*
PM_Rec	mixed herbal tea (fennel seeds, chamomile flowers, peppermint leaves, sage leaves)
* excluded from statistical evaluation	

3.1.3 Homogeneity

The homogeneity for honey and tea samples was tested according to ISO 13528 and the international harmonised protocol for the proficiency testing of analytical chemistry laboratories [9,10].

In order to prove homogeneity, one representative PA which was present in all test samples was selected for honey and tea, respectively. Statistical evaluation was performed for

echimidine in honey and retrorsine in tea to check whether homogeneity criteria were fulfilled. The experimental estimate of sampling standard deviation (s^2_{sam}) has to be below a critical value (*h*), which represents the difference between the experimental estimate of analytical standard deviation and the allowed standard deviation:

$s^2_{sam} = \frac{\left(\frac{V_s}{2} - s^2_{an}\right)}{2}$	$h = F_1\sigma^2_{all} - F_2 s^2_{an}$

where

$$\sigma^2_{all} = (0.3\sigma_t)^2 \qquad V_s = \frac{\sum(S_i - \bar{S})^2}{(i-1)} \qquad \sigma_t = 0.22\bar{S}_{all}$$

$$\bar{S} = \frac{1}{i}\sum S_i \qquad S_i = (a_{i,1} + b_{i,2}) \qquad s^2_{an} = \frac{\sum D_i^2}{2i}$$

$$D_i^2 = (a_{i,1} - b_{i,2})^2 \qquad \bar{S}_{all} = \frac{1}{m}\sum(a_1 + b_{2,} + ...c_m)$$

h:	critical value	$\bar{S}_{all}$:	mean value of all test portions
F_1, F_2:	statistical factors for use in testing for sufficient homogeneity	m:	number of test portions
σ^2_{all}:	allowable sampling variance	i:	number of samples
σ_t:	defined standard deviation	V_s:	variance of S_i
$\bar{S}$:	mean value of S_i	D_i:	difference of each pair of duplicates
S_i:	sum of each pair of duplicates		

Table 3: Critical values (h) and sampling standard deviation (s_{sam}^2) of all test samples

	Critical value (h)	Sampling standard deviation (s_{sam}^2)	Homogeneous if $s_{sam}^2 < h$
HO_01	0.181	0.004	yes
HO_02	0.012	0.001	yes
HO_03	0.089	0.002	yes
HO_04	0.029	0.001	yes
HO_05	0.181	0.004	yes
HO_06	0.033	0.001	yes
PM_01	10.243	2.439	yes
PM_02	6.830	2.095	yes
PM_03	11.044	0.001	yes
PM_04	27.173	4.726	yes
PM_05	10.243	2.439	yes
PM_06	19.398	6.183	yes

All tested materials demonstrated to be sufficiently homogeneous to be used in the method validation study. The results and the statistical evaluation of all samples are given in Annex B and Annex C.

3.1.4 Participants

The list of participants (alphabetical order) of the BfR collaborative study is presented in Table 4.

Table 4: Participants of the collaborative study for the determination of PA in honey and tea

Laboratory	Country
Bavarian national office for Health and Food Safety (LGL Bayern)	Germany
Central Laboratory of the German pharmacists e.V.	Germany
Chemical and Veterinary Analytical Institute Freiburg	Germany
Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL)	Germany
Chemical and Veterinary Analytical Institute Ostwestfalen-Lippe (CVUA-OWL)	Germany
Chemical and Veterinary Analytical Institute Stuttgart Department MT-Toxin Laboratory	Germany
Eurofins WEJ Contaminants GmbH	Germany
Federal Institute for Risk Assessment (BfR)	Germany
Food Safety Laboratory	Singapore
Intertek Food Services GmbH	Germany
National chemical investigation office Rheinland-Pfalz (LUA-RLP)	Germany
National Food Centre	Spain
Nationals Reference Laboratory, Central Institute for Supervising and Testing in Agriculture	Czech Republic
PhytoLab GmbH	Germany
Poisonous Plant Laboratory	USA
Public Analyst's Laboratory	Ireland
Quality Services International GmbH	Germany
RIKILT Wageningen UR	The Netherlands
Service Commun des Laboratoires	France
Technical University of Braunschweig	Germany
The Food and Environment Research Agency	United Kingdom

3.1.5 Study design

3.1.5.1 Samples

All honey and tea samples have been weighed in vessels by the study organiser and were ready to use for the participants of the method validation study.

3.1.5.2 Recovery sample

For the determination of recovery rates, a blank honey sample and a blank tea sample (labelled as recovery sample) were fortified by the participants with 100 µL of the provided PA mixture (labelled as HO_recovery solution for honey and PM_recovery solution for tea). Both fortified samples had to stand at room temperature for five minutes before analysis according to the respective method descriptions for honey and tea.

3.1.5.3 Matrix matched calibration

Matrix matched calibration standard (MMS) solutions covering the following concentration range (150 µL are provided for each MMS level) were prepared and provided by the study organiser:

Honey MMS:

Table 5: Level, labelling and concentration of individual PA of the honey-MMS

Level	Labelling [method description]	Labelling [validation study]	Concentration [vial]	Concentration [honey]
1.	MMS_1	HO_C_1	1 ng/mL	0.1 µg/kg
2.	MMS_2	HO_C_2	5 ng/mL	0.5 µg/kg
3.	MMS_3	HO_C_3	10 ng/mL	1.0 µg/kg
4.	MMS_4	HO_C_4	25 ng/mL	2.5 µg/kg
5.	MMS_5	HO_C_5	50 ng/mL	5.0 µg/kg
6.	MMS_6	HO_C_6	75 ng/mL	7.5 µg/kg
7.	MMS_7	HO_C_7	100 ng/mL	10.0 µg/kg

Tea MMS:

Table 6: Level, labelling and concentration of individual PA in the tea-MMS

Level	Labelling [method description]	Labelling [validation study]	Concentration [vial]	Concentration [tea]
1.	MMS_1	PM_P_1	5 ng/mL	10 µg/kg
2.	MMS_2	PM_C_2	10 ng/mL	20 µg/kg
3.	MMS_3	PM_C_3	25 ng/mL	50 µg/kg
4.	MMS_4	PM_C_4	50 ng/mL	100 µg/kg
5.	MMS_5	PM_C_5	75 ng/mL	150 µg/kg
6.	MMS_6	PM_C_6	100 ng/mL	200 µg/kg
7.	MMS_7	PM_C_7	125 ng/mL	250 µg/kg
8.	MMS_8	PM_C_8	150 ng/mL	300 µg/kg

3.1.5.4 Experimental design

Sequence design
The following sequence design for the LC-MS/MS measurements had to be applied for the honey samples and tea samples, respectively.

1. PA-mixture (in pure solvent)
2. Matrix matched standards (first injection)
3. Solvent blank
4. Samples (first injection)
5. Solvent blank
6. PA-mixture (in pure solvent)
7. Matrix matched standards (second injection)
8. Solvent blank
9. Samples (second injection)

Method
For the preparation of the samples the following two methods had to be used.

- BfR-PA-Honey-1.0_2013
- BfR-PA-Tea-1.0_2013 [1]

[1] updated version: BfR-PA-Tea-2.0_2014

Both methods can be downloaded from:
http://www.bfr.bund.de

English versions:

www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-pa-in-honey.pdf
www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-pa-in-plant-material.pdf

German versions:

www.bfr.bund.de/cm/343/bestimmung-von-pyrrolizidinalkaloiden-in-honig.pdf
www.bfr.bund.de/cm/343/bestimmung-von-pyrrolizidinalkaloiden.pdf

Operating conditions and detection
Participants were asked to optimise chromatographic and mass spectrometric conditions of the laboratory LC-MS/MS system. Analyte detection using triple quadrupole mass spectrometry in multiple reaction monitoring mode was mandatory. An overview of the used operation conditions of each participating laboratory are given in a randomised order in table 7.

Table 7: Overview of the LC-MS/MS operating conditions of the participants

No. of applica-tions	Column	length [mm]	diameter [mm]	particle size [µm]	flow rate [µl/min]	Eluent A	Eluent B	Mass spectrometer
1x	Agilent Zorbrax Eclipse C18	150	2.1	3.5	300	H_2O (0.1 % f.a.)	MeOH (0.1 % f.a.)	Waters Premier
1x	Atlantis C18	100	2.1	3	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5, v/v; 0.1 % f.a.)	Waters TQD
1x	Phenomenex Gemini 5µ	150	3	5	250	H_2O (0.1 % f.a.)	MeOH (0.1 % f.a.)	AB Sciex API 2000
1x	Phenomenex Kinetex PFP	100	2.1	2.6	200	H_2O/MeOH (95/5, v/v; 0.1 % f.a.)	MeOH (0.1 % f.a.)	Varian 320
1x	Phenomenex Kinetex XB	150	2.1	2.6	300	H_2O (0.1 % f.a.)	ACN	Thermo LTQ Velos Pro
1x	Supelco Discovery HSF5	150	2.1	3	250	H_2O (0.1 % f.a.)	ACN (0.1 % f.a.)	AB Sciex 3200 QTrap
2x	Thermo Hypersil Gold	150	2.1	1.9	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5, v/v; 0.1 % f.a.)	AB Sciex API 4000
1x	Thermo Hypersil Gold	150	2.1	1.9	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5, v/v; 0.1 % f.a.)	AB Sciex 5500 QTrap
4x	Thermo Hypersil Gold	150	2.1	1.9	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5, v/v; 0.1 % f.a.)	Thermo TSQ Vantage
1x	Thermo Hypersil Gold	150	2.1	1.9	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5, v/v; 0.1 % f.a.)	Agilent 6410
1x	Waters Atlantis T3	150	2.1	3	400	H_2O (0.1 % f.a.)	MeOH/ACN (50/50, v/v)	Agilent
1x	Waters BEH C18	150	2.1	1.7	200	H_2O (0.1 % f.a.)	ACN (0.1 % f.a.)	Agilent 6410
2x	Waters BEH C18	100	2.1	1.7	300	H_2O (0.1 % f.a.)	MeOH/H_2O (95/5 v/v; 0.1 % f.a.)	Waters XEVO TQ MS
1x	Waters BEH C18	150	2.1	1.7	400	H_2O (0.1 % f.a.)	ACN (0.1 % f.a.)	Waters Quattro premier XE
1x	YMC Triart	150	3	3	350	H_2O (0.1 % f.a.)	MeOH (0.1 % f.a.)	AB Sciex 5500 QTrap
1x	YMC Ultra HAT Hydrosphere C18	75	2	2	200	H_2O (0.1 % f.a.)	MeOH (0.1 % f.a.)	AB Sciex 5500 QTrap

MeOH: methanol; ACN: acetonitrile; f.a.: formic acid

3.1.5.5 Reporting

All participants were requested to report the determined peak areas of the quantifier and the qualifier transition of the 17 PA in a provided excel sheet.

PART A: Honey

3.2 Statistical evaluation of honey data

Statistical analysis of results was carried out following the approach described in the ISO/IUPAC international harmonised protocol, ISO 13528, ISO 5725-2 [9-11].

3.2.1 Preprocessing of honey data

Before starting the statistical evaluation, all submitted values below half of the lowest calibration level (< 0.05 µg/kg) were eliminated. Data sets per sample and individual PA were only evaluated, if at least results of seven laboratories were available. As the isomers intermedine and lycopsamine could not be baseline separated by all participants, the sum of concentrations of both substances was evaluated instead of the individual isomers.

Due to the fact that concentrations resulting from the first and the second injection were calculated using two different calibration curves, the obtained values were considered to be independent values and used for calculation of repeatability standard deviation of the measurement ($RSD_{r,\ measurement}$). The mean value of the first and second measurement of a sample in one laboratory was used for the calculation of the reproducibility standard deviation (between laboratories comparison).

All precision data were evaluated by means of the certified software PROLab® (version 2.15.1.0).

In order to identify conspicuous values, Mandel h and k statistics were applied to both

(a) the variation within the laboratory regarding the twofold measurement (k-statistic) and
(b) the variation between the laboratories (h-statistic) for each sample and for each PA.

If a certain laboratory showed significantly deviating results for one PA over several samples (at a significance level of 5 % for 60 % of the samples or even more or at a significance level of 1 % for 50 % of the samples), it was considered as a conspicuous laboratory and results of the respective analyte were eliminated for all samples. Furthermore, if for one sample a laboratory showed significantly deviating results over several analytes (at a significance level of 5 % for 60 % of the analytes or even more or at a significance level of 1 % for 50 % or more of the analytes) it was considered as a conspicuous laboratory and all results of the laboratory for this sample were eliminated. For submitted results including the Mandel h- and k-statistic-diagrams please refer to Annex F.

The following tables summarise the data sets which were eliminated prior to further statistical analysis.

Lab_024 reported technical problems during the entire measurement; therefore no results were evaluated.

Lab_025 participated in the method validation for tea only.

Table 8: Eliminated samples per laboratory

	HO_04	HO_06	HO_26
LC_009	(5/3/1)h		
LC_016		(5/2/2)h	(5/4/-)k
LC_019		(3/2/-)h	
(A/B/C): A: Number of detected analytes in the sample B: Number of conspicuous values at 1 % significance level C: Number of conspicuous values at 5 % significance level k: Conspicuous sample due to within laboratory (k-statistic) h: Conspicuous sample due to between laboratories (h-statistic)			

Table 9: Eliminated analytes for all honey samples

Lab.-code	Echimidine	Retrorsine
LC_015	(8/4/3)h	
LC_016		(7/5/-)k
(A/B/C): A: Number of the detected analytes in all samples B: Number of conspicuous values at 1 % significance level C: Number of conspicuous values at 5 % significance level k: Conspicuous analyte due to within laboratory (k-statistic h: Conspicuous analyte due to between laboratories (h-statistic)		

3.2.2 Calculation of the precision data in honey

To characterise the precision of the method the repeatability standard deviation and the reproducibility standard deviation were calculated in PROLab® (version 2.15.1.0). Respective relative parameters like the 'relative repeatability standard deviation' (RSD_r) and 'relative reproducibility standard deviation' (RSD_R) were calculated to allow comparison between different analytes and test samples.

Recalculation of the Mandel h and k statistics did not reveal further conspicuous values. Consequently, results were ready to be checked for outliers applying Cochran and Grubbs test.

The *Cochran test* is used to identify laboratories with significantly high deviation between results of first and second measurement (within-laboratory comparison) with a statistical significance of 5 %.
The *Grubbs test* is used to identify laboratories which reported results with a significant deviation from the mean value calculated from the first and second measurement value of all laboratories (between laboratories comparison) with a statistical significance of 5 %.

3.2.3 Calculation of the HorRat value for the honey data

The Horwitz Ratio (HorRat value) describes the ratio between the determined reproducibility standard deviation and the predicted reproducibility standard deviation, which is calculated from the Horwitz equation [12,13].

$HORRAT = \frac{RSD_R}{PRSD}$	$PRSD = 2^{\left(1-\frac{\log M_R}{2}\right)}$

where

RSD_R: reproducibility standard deviation M_R: normalised mean value [e.g. kg/kg]

$PRSD$: predicted standard deviation

The HorRat value is used as a measure to determine the sufficiency of the precision of a method. In this context the following guidelines are commonly applied:

HORRAT ≤ 0.5:	Method reproducibility may be in question due to lack of study Independence, unreported averaging or consultation
HORRAT ≤ 1.5	Method reproducibility as normally would be expected
HORRAT > 1.5	Method reproducibility higher than normally expected
HORRAT > 2.0	Method reproducibility is problematic

In total, six different honey samples labelled as HO_01 to HO_06 had to be analysed. HO_02 and HO_06 were blind duplicates and were additionally evaluated as HO_26. For the determination of the recovery rate a PA- free honey was spiked by the participants with a PA-mixture (containing 17 PA dissolved in water/methanol [95/5, v/v]) subsequently analysed. The final spiking concentration was 5.0 µg individual PA/kg honey (15 µg McN/kg honey).

The statistical evaluation including precision data of the individual honey samples are given in the following tables.

3.2.4 Performance characteristics for the recovery sample (honey)

Table 10: Performance characteristics for the determination of 17 PA in honey (recovery sample)

	Em	Hn	Lc	Mc	Re	Sc	Sk	Sp	Td	HnN	LcN	McN	ReN	ScN	SpN	Im/La
n of accepted results	20	20	20	20	20	20	20	20	20	20	20	19	20	20	20	20
outlier	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4
n of evaluated results	19	20	20	20	20	20	20	20	20	20	20	19	19	20	20	16
mean [µg/kg]	4.3	4.2	4.0	3.8	4.7	4.0	5.0	3.8	3.9	5.2	4.3	12.2	4.7	4.8	3.3	7.4
fortified value [µg/kg]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	15.0	5.0	5.0	5.0	10.0
$RSD_{r,\ measurement}$ [%]	3.9	4.7	3.9	5.8	9.6	5.4	4.3	7.0	5.4	4.2	6.0	10.4	5.7	7.8	7.7	3.3
RSD_R, [%]	20.8	23.9	17.7	42.1	24.8	21.6	18.9	27.6	25.3	21.8	19.9	33.1	24.6	23.6	44.9	17.2
$PRSD_R$ (Horwitz)	36.4	36.5	36.7	37.1	36.9	36.8	35.5	37.0	36.9	36.3	36.3	31.1	35.8	35.7	37.9	33.5
HorRat	**0.6**	**0.7**	**0.5**	**1.1**	**0.7**	**0.6**	**0.5**	**0.7**	**0.7**	**0.6**	**0.5**	**1.1**	**0.7**	**0.7**	**1.2**	**0.5**
recovery [%]	**86**	**84**	**80**	**75**	**94**	**79**	**100**	**77**	**78**	**103**	**87**	**81**	**95**	**96**	**65**	**74**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.5 Performance characteristics for HO_01 (blossom honey)

Table 11: Performance characteristics for HO_01 (blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	18	17	19	11	19
outliers	0	0	0	0	1
n of evaluated results	18	17	19	11	18
mean [µg/kg]	4.4	1.9	1.0	0.6	2.9
$RSD_{r,\ measurement}$ [%]	5.3	8.4	9.5	10.6	7.3
RSD_R, [%]	19.7	24.8	27.3	20.6	54.3
$PRSD_R$ (Horwitz)	36.2	41.3	45.2	49.3	38.5
HorRat	**0.5**	**0.6**	**0.6**	**0.4**	**1.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.6 Performance characteristics for HO_02 (summer blossom honey)

Table 12: Performance characteristics for HO_02 (summer blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	19	18	20	20	20
outliers	1	0	0	0	1
n of evaluated results	18	18	20	20	19
mean [µg/kg]	1.1	3.2	2.1	1.1	4.2
$RSD_{r,\ measurement}$ [%]	3.9	7.4	6.6	10.2	9.9
RSD_R, [%]	22.4	26.4	27.9	31.3	51.1
$PRSD_R$ (Horwitz)	44.5	38.0	40.5	44.5	36.5
HorRat	**0.5**	**0.7**	**0.7**	**0.7**	**1.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.7 Performance characteristics for HO_03 (blossom honey)

Table 13: Performance characteristics for HO_03 (blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	19	18	20	20	20
outliers	0	0	1	1	0
n of evaluated results	19	18	19	19	20
mean [µg/kg]	3.0	0.9	1.6	1.1	4.1
$RSD_{r,\ measurement}$ [%]	5.2	16.9	10.2	8.1	9.8
RSD_R, [%]	21.4	28.0	27.1	30.7	26.2
$PRSD_R$ (Horwitz)	38.4	46.1	42.2	44.9	36.6
HorRat	**0.6**	**0.6**	**0.6**	**0.7**	**0.7**

n: number
R $RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.8 Performance characteristics for HO_04 (blossom honey)

Table 14: Performance characteristics for HO_04 (blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	17	16	18	18	18
outliers	0	0	0	0	1
n of evaluated results	17	16	18	18	17
mean [µg/kg]	1.0	3.0	5.4	2.7	2.3
$RSD_{r,\ measurement}$ [%]	8.1	8.8	7.4	8.9	6.6
RSD_R, [%]	30.7	23.3	32.8	32.7	34.4
$PRSD_R$ (Horwitz)	41.4	38.5	35.1	38.9	40.0
HorRat	**0.7**	**0.6**	**0.9**	**0.8**	**0.9**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.9 Performance characteristics for HO_05 (orange blossom honey)

Table 15: Performance characteristics for HO_05 (orange blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	18	17	19	19	19
outliers	0	1	0	0	0
n of evaluated results	18	16	19	19	19
mean [µg/kg]	1.9	8.4	16.9	8.7	4.6
$RSD_{r,\ measurement}$ [%]	6.8	9.7	6.5	5.6	11.0
RSD_R, [%]	18.7	32.0	26.9	33.8	51.6
$PRSD_R$ (Horwitz)	41.1	32.8	29.6	32.7	36.0
HorRat	**0.5**	**1.0**	**0.9**	**1.0**	**1.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.10 Performance characteristics for HO_06 (summer blossom honey)

Table 16: Performance characteristics for HO_06 (summer blossom honey)

	Em	Re	Sc	Sp	Im/La
n of accepted results	17	17	18	18	18
outliers	1	0	0	1	0
n of evaluated results	16	17	18	17	18
mean [µg/kg]	1.1	3.3	2.2	1.1	4.0
$RSD_{r,\ measurement}$ [%]	5.7	10.3	7.0	6.8	18.0
RSD_R, [%]	17.0	17.6	20.7	28.6	61.0
$PRSD_R$ (Horwitz)	44.6	37.7	40.3	44.4	36.7
HorRat	**0.4**	**0.5**	**0.5**	**0.6**	**1.7**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.11 Performance characteristics for HO_26 (blind duplicate)

Table 17: Performance characteristics for HO_26 (blind duplicate)

	Em	Re	Sc	Sp	Im/La
n of accepted results	36	36	38	37	38
outliers	1	2	0	0	0
n of evaluated results	35	34	38	37	38
mean [µg/kg]	1.1	3.3	2.1	1.1	4.2
$RSD_{r,}$ [%]	5.9	9.2	10.5	6.7	12.7
$RSD_{R,}$ [%]	20.0	22.6	28.0	29.1	52.1
$PRSD_R$ (Horwitz)	44.5	37.9	40.6	44.4	36.5
HorRat	**0.4**	**0.6**	**0.7**	**0.7**	**1.4**

n: number
$RSD_{r,}$: relative repeatability standard deviation
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.2.12 Discussion

After removal of the outliers indicated by Cochran and Grubb test, the relative reproducibility standard deviations (RSD_R), the relative repeatability standard deviations of duplicate measurement ($RSD_{r,\ measurement}$), the relative repeatability standard deviation for the blind duplicate (RSD_r) and the Horwitz ratio (HorRat) were calculated for each detected PA (refer to Table 10-17).

3.2.12.1 Recovery sample (HO_recovery)

The $RSD_{r,\ measurement}$ ranged between 3.9 % (echimidine) and 10.4 % (monocrotaline-N-oxide) with obtained RSD_R values between 17.7 % (lasiocarpine) and 44.9 % (seneciphylline-N-oxide). Resulting HorRat values were between 0.5 (lasiocarpine) and 1.2 (seneciphylline-N-oxide).

This indicates a high precision of the applied method and good comparability of the results in different laboratories for a honey sample fortified with a PA standard solution.

According to AOAC Guidelines for standard method performance requirements, the obtained recovery, which ranged between 75 % for monocrotaline and 103 % for heliotrine-N-oxide are considered to be sufficient for reliable analysis [14].

3.2.12.2 Honey samples (HO_01-HO_06)

Excellent repeatability and reproducibility precision were achieved for the six honey samples which contained echimidine, retrorsine, senecionine, seneciphylline and the isomers intermedine and lycopsamine (please refer to Table 11 to Table 16).

The $RSD_{r,\ measurement}$ ranged between 3.9 % (echimidine in HO_02; Table 12) and 18 % (sum of intermedine and lycopsamine in HO_06; Table 16).

The RSD_R ranged between 17 % (echimidine in HO_06; Table 16) and 33.8 % (seneciphylline in HO_05; Table 15), whereas considerably higher values were obtained for the sum of lycopsamine and intermedine (up to 61 % in HO_06; Table 16). Resulting HorRat values

ranged between 0.4 (seneciphylline in HO_01; Table 11) and 1.0 (retrorsine in HO_05; Table 15). For the sum of intermedine and lycopsamine HorRat values were between 0.7 (HO_03; Table 13) up to 1.7 (HO_06; Table 16).

Compared to the fortified recovery sample the obtained HorRat values for the sum of intermedine and lycopsamine of naturally contaminated samples are higher. A possible explanation might be that naturally contaminated samples contained more isomers than lycopsamine and intermedine. Chromatrographic separation achieved in different laboratories varies as some laboratories do not achieve baseline separation of intermedine and lycopsamine and others are even able to chromatographically separate further lycopsamine isomers. Therefore, the sum of lycopsamine and intermedine signals may arise from a different number of individual toxins resulting in a lower reproducibility precision.

3.2.12.3 Blind Duplicate (HO_26)

The investigated samples HO_02 and HO_06 were a blind duplicate and were additionally evaluated as HO_26 (please refer to Table 17) to determine repeatability.

The repeatability standard deviation (RSD_r) of a twofold analysis (including sample preparation, clean-up and measurement) was calculated and ranged between 5.9 % for echimidine to 12.7 % for the sum of intermedine and lycopsamine. Compared to the relative repeatability SD of measurement ($RSD_{r,\ measurement}$ of HO_02 (Table 12) and HO_06 (Table 16) ranging from 3.9 % for echimidine to 12.7 % for the sum of intermedine and lycopsamine) the obtained values were in the same range. This demonstrates that the sample preparation, including the SPE clean-up, has less influence on the methods precision than the LC-MS/MS measurement.

Therefore, it can be concluded that results obtained from a twofold measurement as well as results obtained from a twofold analysis reveal a comparable precision.

Although, a twofold measurement did not increase the methods precision it is recommended unless some other quality control is applied. As already shown, the sample preparation step has less influence on the methods precision than the LC-MS/MS measurement. Therefore, a high deviation in duplicate measurement can reveal problems during analysis and provides a powerful tool to ensure quality of analysis.

PART B: Tea

3.3 Statistical evaluation of tea data

Statistical analysis of results was carried out following the approach described in the ISO/IUPAC international harmonised protocol, ISO 13528, ISO 5725-2 [9-11].

3.3.1 Preprocessing of tea data

Before starting statistical evaluation, all reported values below half of the lowest calibration level (< 5 µg/kg) were eliminated. Data sets per sample and individual PA were only evaluated, if at least results of seven laboratories were available after elimination of reported concentrations below 5 µg/kg. As the isomers intermedine and lycopsamine could not be baseline separated by all participants, the sum of concentrations of both substances was evaluated instead of the individual isomers.

Due to the fact that concentrations resulting from the first and the second injection were calculated using two different calibration curves, the obtained values were considered to be independent values and used for calculation of repeatability standard deviation of duplicate measurement ($RSD_{r,\ measurement}$). The mean value of the first and second measurement per laboratory was calculated and used for the calculation of the reproducibility standard deviation (between laboratories comparison).

All precision data were evaluated by means of the certified software PROLab® (version 2.15.1.0)

In order to identify conspicuous values Mandel h and k statistics were applied to both

(a) the variation within the laboratory regarding the twofold measurement (k-statistic) and
(b) the variation between the laboratories (h-statistic) for each sample and for each PA.

If a certain laboratory showed significantly deviating results for one PA over several samples (at a significance level of 5 % for 60 % of the samples or even more or at a significance level of 1 % for 50 % of the samples), it was considered to be a conspicuous laboratory and results of the respective analyte were eliminated for all samples. Furthermore, if for one sample a laboratory showed significantly deviating results over several analytes (at a significance level of 5 % for 60 % of the analytes or even more or at a significance level of 1 % for 50 % or more of the analytes), it was considered to be a conspicuous laboratory and all results of the laboratory for this sample were eliminated. For submitted results including the Mandel h and k statistic diagrams please refer to Annex F.
The following tables summarise the data sets which were eliminated from further statistical analysis.

Lab_001 reported technical problems during the measurement of the tea recovery sample (PM_recovery), therefore this sample was not evaluated.
Lab_024 reported technical problems during the entire measurement; therefore the results were not included in the evaluation.

Table 18: Eliminated samples per laboratory

	PM_recovery
LC_012	(18/13/1)k
(A/B/C): A: number of detected analytes in the sample; B: number of conspicuous values at 1 % significance level; C: number of conspicuous values at 5 % significance level k: conspicuous sample due to within the laboratory (k statistic) h: conspicuous sample due to between the laboratories (h statistic)	

Table 19: Eliminated analytes for all tea samples

Lab.-code	echimidine	heliotrine	heliotrine-N-oxide	lasiocarpine	lasiocarpine-N-oxide	senkirkine	retrorsine	retrorsine-N-oxide	seneciphylline-N-oxide
LC001			(4/1/2)h						
LC002				(4/3/-)h					
LC003						(2/2/-)h			
LC005					(4/2/2)h				
LC012	(2/2/-)k	(4/3/-)k				(2/1/1)k	(8/6/1)h (8/7/-)k		
LC103									(2/1/1)k
LC118								(8/3/3)k	
(A/B/C): A: number of detected analytes in all sample; B: number of conspicuous values at 1 % significance level; C: number of conspicuous values at 5 % significance level k: conspicuous analyte due to within the laboratory (k statistic) h: conspicuous analyte due to between the laboratories (h statistic)									

3.3.2 Calculation of the precision data in tea

To characterise the precision of the method the repeatability standard deviation and the reproducibility standard deviation were calculated in PROLab® (version 2.15.1.0). Respective relative parameters like the 'relative repeatability standard deviation' (RSD_r) and 'relative reproducibility standard deviation' (RSD_R) were calculated to allow comparison between different analytes and test samples.

Recalculating the Mandel h and k statistics did not reveal further conspicuous results. Consequently, outliers were identified applying Cochran and Grubbs' test.

The *Cochran test* is used to identify laboratories with significantly high deviation between results of first and second measurement (within-laboratory comparison) with a statistical significance of 5 %.

The *Grubbs' test* is used to identify laboratories which reported results with a significant deviation from the mean value calculated from the first and second measurement value of all laboratories (between laboratory comparison) with a statistical significance of 5 %.

3.3.3 Calculation of the HorRat value for the tea data

The Horwitz Ratio (HorRat value) describes the ratio between the reproducibility standard deviation and the predicted reproducibility standard deviation, which is calculated from the Horwitz equation [12,13].

$HORRAT = \frac{RSD_R}{PRSD}$	$PRSD = 2^{\left(1-\frac{\log M_R}{2}\right)}$

where

RSD_R: reproducibility standard deviation

$PRSD$: predicted standard deviation

M_R: normalised mean value [e.g. kg/kg]

The HorRat value is used as a measure to determine the sufficience of the precision of a method. In this context the following guidelines are commonly applied.

HORRAT ≤ 0.5:	Method reproducibility may be in question due to lack of study independence, unreported averaging or consultation
HORRAT ≤ 1.5	Method reproducibility as normally would be expected
HORRAT > 1.5	Method reproducibility higher than normally expected
HORRAT > 2.0	Method reproducibility is problematic

In total six different tea samples labelled as PM_01 to PM_06 had to be analysed, where PM_01 and PM_05 were blind duplicates and were additionally evaluated as PM_15. For the determination of recovery rates a PA- free mixed herbal tea was spiked by the participants with a PA-mixture (containing 17 PA dissolved in water/methanol [95/5, v/v]) subsequently analysed.

The statistical evaluation including precision data of the individual tea samples are given in the following tables.

3.3.4 Performance characteristics for the recovery sample (PM_recovery)

Table 20: Performance characteristics for the determination of 17 PA in tea (recovery sample)

	Em	Hn	Lc	Mc	Re	Sc	Sk	Sp	Td	HnN	LcN	McN	ReN	ScN	SpN	Im/La
n of accepted results	19	19	18	19	19	19	18	19	19	19	18	19	17	19	18	19
outliers	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
n of evaluated results	19	19	18	19	19	19	18	18	19	19	18	19	17	18	18	19
mean [µg/kg]	**143**	**130**	**114**	**122**	**103**	**105**	**125**	**119**	**120**	**150**	**114**	**122**	**128**	**116**	**110**	**270**
fortified value [µg/kg]	**150**	**150**	**150**	**150**	**100**	**100**	**100**	**100**	**150**	**150**	**100**	**150**	**150**	**100**	**100**	**300**
$RSD_{r,\ measurement}$ [%]	6.6	6.1	7.0	8.4	8.1	8.1	6.2	9.8	6.2	7.1	7.0	7.5	5.7	8.2	8.9	7.1
$RSD_{R,}$ [%]	25.7	24.8	18.9	19.6	22.9	23.1	23.5	27.3	19.5	21.6	18.4	18.6	24.4	21.3	19.3	22.1
$PRSD_R$ (Horwitz)	21.4	21.7	22.2	22.0	22.5	22.5	21.9	22.0	22.0	21.3	22.2	22.0	21.8	22.1	22.3	19.5
HorRat	**1.2**	**1.1**	**0.9**	**0.9**	**1.0**	**1.0**	**1.1**	**1.2**	**0.9**	**1.0**	**0.8**	**0.8**	**1.1**	**1.0**	**0.9**	**1.1**
recovery [%]	**95**	**87**	**76**	**81**	**103**	**105**	**125**	**119**	**80**	**100**	**114**	**81**	**85**	**116**	**110**	**90**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.5 Performance characteristics for PM_01 (melissa tea)

Table 21: Performance characteristics for PM_01 (melissa tea)

	Hn	Lc	Re	Sc	Sp	HnN	LcN	ReN	ScN	SpN	Im/La
n of accepted results	19	16	17	20	17	19	20	19	21	19	13
outliers	0	0	0	0	0	0	0	1	0	0	2
n of evaluated results	19	16	17	20	17	19	20	18	21	19	11
mean [µg/kg]	31.0	11.5	18.6	33.3	34.9	159.7	45.4	83.2	108.0	63.7	9.0
$RSD_{r,\ measurement}$ [%]	6.6	19.7	16.6	14.3	7.9	4.2	7.9	4.2	8.5	10.7	15.2
RSD_R [%]	32.0	37.7	45.0	40.6	33.8	31.3	38.1	32.2	27.2	31.9	39.1
$PRSD_R$ (Horwitz)	27.0	31.3	29.1	26.7	26.5	21.1	25.5	23.3	22.4	24.2	32.5
HorRat	**1.2**	**1.2**	**1.5**	**1.5**	**1.3**	**1.5**	**1.5**	**1.4**	**1.2**	**1.3**	**1.2**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.6 Performance characteristics for PM_02 (chamomile tea)

Table 22: Performance characteristics for PM_02 (chamomile tea)

	Em	Re	Sc	Sk	Sp	ReN	ScN	SpN	Im/La
n of accepted results	14	16	20	17	17	19	21	20	20
outliers	0	0	0	0	0	1	0	0	0
n of evaluated results	14	16	20	17	17	18	21	20	20
mean [µg/kg]	26.2	15.5	80.4	17.6	36.4	95.0	348	165	56.3
$RSD_{r,\ measurement}$ [%]	10.2	16.7	9.5	12.0	8.5	5.1	5.1	6.8	10.6
RSD_R [%]	67.0	26.9	37.1	41.9	33.1	24.2	28.6	20.1	35.2
$PRSD_R$ (Horwitz)	27.7	30.0	23.4	29.4	26.3	22.8	18.7	21.0	24.7
HorRat	**2.4**	**0.9**	**1.6**	**1.4**	**1.3**	**1.1**	**1.5**	**1.0**	**1.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.7 Performance characteristics for PM_03 (mixed herbal tea)

Table 23: Performance characteristics for PM_03 (mixed herbal tea)

	Re	Sc	ReN	ScN	Im/La
n of accepted results	18	20	19	20	18
outliers	0	0	0	0	1
n of evaluated results	18	20	19	20	17
mean [µg/kg]	22.4	48.4	33.4	76.0	34.4
$RSD_{r,\ measurement}$ [%]	14.7	10.8	16.1	7.0	17.3
RSD_R [%]	41.2	40.3	40.5	38.4	56.2
$PRSD_R$ (Horwitz)	28.3	25.2	26.7	23.6	26.6
HorRat	**1.5**	**1.6**	**1.5**	**1.6**	**2.1**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.8 Performance characteristics for PM_04 (rooibos tea)

Table 24: Performance characteristics for PM_04 (rooibos tea)

	Re	Sc	ReN	ScN
n of accepted results	18	20	19	21
outliers	0	0	0	0
n of evaluated results	18	20	19	21
mean [µg/kg]	32.7	74.5	102	287
$RSD_{r,\ measurement}$ [%]	11.1	11.2	7.0	7.0
RSD_R [%]	51.6	43.7	44.8	46.8
$PRSD_R$ (Horwitz)	26.8	23.7	22.5	19.3
HorRat	**1.9**	**1.8**	**2.0**	**2.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.9 Performance characteristics for PM_05 (melissa tea)

Table 25: Performance characteristics for PM_05 (melissa tea)

	Hn	Lc	Re	Sc	Sp	HnN	LcN	ReN	ScN	SpN	Im/La
n of accepted results	19	15	18	20	19	20	20	19	21	19	10
outliers	0	0	0	0	0	0	0	0	0	0	0
n of evaluated results	19	15	18	20	19	20	20	19	21	19	10
mean [µg/kg]	33.7	11.6	20.8	34.6	34.2	155	45.7	77.5	108	65.5	17.3
$RSD_{r,\ measurement}$ [%]	9.4	24.0	17.3	13.7	13.5	8.0	11.3	8.1	7.3	11.3	14.3
RSD_R [%]	38.8	28.2	34.4	38.1	38.3	28.9	33.0	23.4	32.1	27.0	70.7
$PRSD_R$ (Horwitz)	26.7	31.3	28.7	26.5	26.6	21.2	25.5	23.5	22.4	24.1	29.5
HorRat	**1.5**	**0.9**	**1.2**	**1.4**	**1.4**	**1.4**	**1.3**	**1.0**	**1.4**	**1.1**	**2.4**

n: number
$RSD_{r,\ measurement}$: relative repeatability standard deviation of measurement
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.10 Performance characteristics for PM_15 (blind duplicate)

Table 26: Performance characteristics for PM_15 (blind dublicate)

	Hn	Lc	Re	Sc	Sp	HnN	LcN	ReN	ScN	SpN	Im/La
n of accepted results	37	29	33	39	36	38	40	38	42	38	15
outliers	0	0	0	0	0	0	0	0	0	0	4
n of evaluated results	37	29	33	39	36	38	40	38	42	38	11
mean [µg/kg]	32.3	11.6	19.7	34.3	34.5	155.9	45.5	80.4	108.1	64.6	10.8
RSD_r [%]	32.2	24.5	21.1	14.4	11.2	26.5	21.1	16.8	16.0	13.8	38.7
RSD_R [%]	35.6	29.5	37.3	36.9	36.1	29.1	34.8	27.6	29.1	28.4	38.7
$PRSD_R$ (Horwitz)	26.8	31.3	28.9	26.6	26.6	21.2	25.5	23.4	22.4	24.2	31.6
HorRat	**1.3**	**0.9**	**1.3**	**1.4**	**1.4**	**1.4**	**1.4**	**1.4**	**1.3**	**1.2**	**1.2**

n: number
$RSD_{r,\ measurement}$: relative repeatability measurement standard deviation
RSD_R: relative reproducibility standard deviation
$PRSD_R$: predicted standard deviation according to Horwitz
HorRat: Horwitz ratio

3.3.11 Discussion

After removal of the outliers indicated by Cochran and Grubbs' test, the relative reproducibility standard deviation (RSD_R), the relative measurement repeatability standard deviation ($RSD_{r,\ measurement}$), the relative repeatability standard deviation for the blind duplicate (RSD_r) and the Horwitz ratio (HorRat) were calculated for each detected PA (please refer to Table 20).

3.3.11.1 Recovery sample (PM_recovery)

$RSD_{r,\ measurement}$ ranged between 5.7 % (retrorsine-N-oxide) and 9.8 % (seneciphylline) and RSD_R ranged between 18.4 % (lasiocarpine-N-oxide) and 27.3% (seneciphylline). Based on the consideration of the calculated predicted standard deviation according to Horwitz, HorRat values were achieved between 0.8 for lasiocarpine-N-oxide and 1.2 for seneciphylline.
This indicates a sufficient precision and a good inter-laboratory comparability of the applied method for the detection of PA in a tea sample fortified with a PA standard solution.

According to AOAC Guidelines for standard method performance requirements, the obtained recoveries, which range between 76 % for lasiocarpine and 125 % for senkirkine, are considered to be sufficient for reliable analysis [14].

3.3.11.2 Tea samples (PM_01-PM_05)

Sufficient reproducibility precision was achieved for the five naturally contaminated tea samples which contained different amounts of 14 of the 17 investigated PA. Monocrotaline, monocrotaline-N-oxide and trichodesmine were not present in the samples.

The $RSD_{r,\ measurement}$ ranged between 4.2 % (retrorsine-N-oxide in melissa tea [PM_01; Table 21]) and 24.0 % (lasiocarpine in melissa tea [PM_05; Table 25]).The RSD_R values obtained for the tea samples were higher than for the honey samples, which can, amongst other things, probably be explained by the more complex matrix tea. The RSD_R ranged from 23.4 % (retrorsine-N-oxide in melissa tea [PM_05; Table 25]) to 67.0 % (echimidine in chamomile tea [PM_02; Table 22]. Even higher RSD_R were obtained for the sum of lycopsamine and intermedine (up to 70.7 % in melissa tea [PM_05; Table 25]). These partially high values deviate from the excellent RSD_R obtained for the tea recovery sample (Table 20). RSD_R values for the recovery sample for tea were in the same range as for honey. The high deviations could be caused by an inhomogeneous distribution of the respective PAs. This seems obvious for the PA diester echimidine in PM_02 (Table 22). The calculated $RSD_{r,\ measurement}$ of 10.2 % is acceptable and in the same range as for other analytes, but the corresponding RSD_R with 67 % is extremely high (resulting in a HorRat value of 2.4). Furthermore, it has also to be assumed that naturally contaminated tea samples contain a varying number of PA isomers. Different isomer separation efficiencies of the laboratories will lead inevitably to deviating results. Since the participating laboratories used different chromatographic conditions it cannot be excluded that echimidine and further echimidine isomers coeluted unnoticed (one lab reported the presence of heliosupine) leading to different results. Interestingly, for the macrocyclic ester retrorsine, which is also present in PM_02 (Table 22), an adequate RSD_R value of 26.9 % was obtained resulting in an excellent HorRat value of 0.9. Therefore, it has to be considered, that the distribution of individual PA can be different within the subsamples of one sample. This can be explained by the fact that individual PA may originate either from different PA-producing plants or from different parts of one plant (e.g. seeds, leaves, flowers, stems) [15]. For instance, the open chained diester echimidine and the macrocyclic diester retrorsine (both present in PM_02) cannot be synthesised by the same plant. Therefore, a contamination by several plants has to be considered. Different physical properties of the plants or parts of plants may lead to a varying distribution of PA during milling and homogenisation process.

The issues described seem to be also present for the PA determined in PM_03 (mixed herbal tea; Table 23) and PM_04 (rooibos tea; Table 24). While $RSD_{r,\ measurement}$ values between 7.0 % and 17.3 % were acceptable, RSD_R values were relatively high (38.4 % and 56.2 %), although the homogeneity of retrorsine had been proven beforehand. It has to be considered,

that compliance of retrorsine concentrations in homogeneity test samples was achieved by chance.

3.3.11.3 Blind Duplicate (PM_15)

The investigated samples PM_01 and PM_05 were blind duplicates and were additionally evaluated as PM_15 (please refer to Table 26).

In contrast to the obtained $RSD_{r,\ measurement}$ (twofold measurement) of the analysed tea samples PM_01 to PM_05 (mean: 10 %), the calculated relative repeatability SD (RSD_r) of a twofold analysis (including sample preparation, clean-up and measurement) was considerably higher and ranged between 14 % (seneciphylline-N-oxide) and 39 % (sum of intermedine/lycopsamine).

The evaluation of the blind duplicate showed no difference between RSD_R and the RSD_r. Usually, the repeatability is approx. ½ to ⅔ of the reproducibility, which is also the case for the determination of PA in honey (table 26) [16]. Those results confirm the assumption that PA were probably inhomogeneously distributed in the melissa tea sample used as blind duplicate.

These findings demonstrate the need of an optimised homogenisation process and sampling step for tea samples. For example it is possible to increase the used amount of sample and the extraction volume, while keeping the same sample/extraction-volume ratio (e.g. 10 g/200 ml). Another possibility is the reduction of the particle size. Both options would take into account the different physical properties and the resulting differences in distribution of the divers PA plant parts (e.g. seeds, flowers, stems, leaves) in a naturally contaminated tea sample.

In general, the results of the study indicate that the method performance characteristics were mostly sufficient with few exceptions due to the issue of homogeneity. However, excellent results obtained for the recovery sample in tea (PM_recovery) proved the method is applicability to determine PA in tea.

4 Conclusions

The study, with 20 participating laboratories, comprised the determination of 17 pyrrolizidine alkaloids in six naturally contaminated honey and six tea samples. Both sample sets further included a recovery sample and a blind duplicate. As the clean-up method for the determination of PA in black and green tea was modified evaluation of the results for PM_06 (black tea) was refrained from. For the updated method protocol, please refer to www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-in-plant-material.pdf *(English version)* *or* www.bfr.bund.de/cm/343/bestimmung-von-pyrrolizidinalkaloiden.pdf *(German version).*

The results for honey showed low intra-laboratory standard deviation for repeated measurement ($RSD_{r,\ measurement}$) and for repeated analysis (RSD_r), as well as low inter-laboratory standard deviation (RSD_R). The HorRat values of 17 PA for the honey recovery sample (fortified with 5-15 µg/kg) ranged between 0.5 and 1.2 with recovery rates between 75 % and 103 %. The naturally contaminated honey samples contained echimidine, retrorsine, senecionine, seneciphylline and the isomers intermedine and lycopsamine in a concentration range from 0.6 µg/kg up to 16.9 µg/kg. The obtained HorRat values for the naturally contaminated honey samples were in a satisfactory range from 0.4 to 1.7 and in the same range as the recovery sample. Furthermore, the comprehensive evaluation of the blind duplicate sample demonstrated a similar precision of results achieved from a twofold analysis (two independent sample preparation including clean-up and measurement; RSD_r: 5.9 % - 12.7 %) and the results achieved from twofold measurement (twofold measurement of the same sample obtained from two independent calibrations; $RSD_{r,\ measurement}$: 3.9-18.3 %). This indicates that imprecision of the method for honey analysis is dominated by LC-MS/MS detection and not by the sample preparation. Therefore, it can be concluded that the method is applicable for the determination of PA in honey.

In general, analysis of tea samples showed higher intra-laboratory standard deviation ($RSD_{r,\ measurement}$) and inter-laboratory standard deviation (RSD_R) than analysis in honey. The HorRat values of 17 PA for the tea recovery sample (fortified with 100-150 µg/kg) ranged between 0.8 and 1.2 with recovery rates between 76 % and 125 %. These data indicate a sufficient precision and a good inter-laboratory comparability with acceptable recovery rates of the applied method. The five naturally contaminated tea samples contained 14 of the 17 investigated PA (except Td and Mc, McN) in a concentration range of 9 µg/kg to 348 µg/kg. The obtained HorRat values for the naturally contaminated tea samples were in a range from 0.9 to 2.4 and are higher than for the recovery sample. As the precision of duplicate measurement obtained for the recovery sample and the naturally contaminated sample were in the same range it has to be assumed that the higher RSD_R values of naturally contaminated samples indicates an inhomogeneous distribution of PA (regardless the demonstrated homogeneity for retrorsine). Further, an insufficient chromatographic separation of PAs with a varying number of naturally occurring isomers may result in an incorrect quantification. Both assumptions are supported by the fact that repeatability and reproducibility SD of the blind duplicate were similar although a lower repeatability has to be expected. Consequently, the sample pretreatment step (milling and homogenisation) should be optimised with regard to homogeneity but the chromatographic separation should also be taken into account. The updated method protocol includes two possible options to limit the influence of inhomogeneity, for instance by the use of larger sample amounts or by the reduction of the particle size. It is up to the laboratories to prove their homogenisation method to be sufficient.

It can be concluded that, due to the excellent measurement precision and the satisfactory performance characteristics obtained for the recovery sample, the method is applicable for the determination of PA in tea.

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Annex B Homogeneity data for echimidine in honey

HO_01 und HO_05 (blossom honey):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	4.78	4.66	9.44	0.013
2	4.70	4.74	9.44	0.002
3	4.79	4.65	9.44	0.020
4	4.73	4.71	9.45	0.000
5	4.75	4.70	9.45	0.002
6	4.59	4.51	9.09	0.006
7	4.53	4.55	9.07	0.0003
8	4.67	4.71	9.38	0.001
9	4.67	4.71	9.37	0.002
10	4.67	4.66	9.33	0.0001
Cochran test			Grubbs' test	
C:	0.403		G:	2.170
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				0.181
s^2_{sam}				0.004
sufficient homogeneous if critical value > s^2_{sam}			accepted	

HO_02 (summer blossom honey:

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	1.25	1.25	2.51	0.000
2	1.21	1.18	2.39	0.001
3	1.18	1.17	2.35	0.00006
4	1.18	1.18	2.36	0.00003
5	1.24	1.20	2.44	0.002
6	1.23	1.20	2.43	0.001
7	1.13	1.17	2.30	0.0020
8	1.23	1.16	2.40	0.005
9	1.19	1.16	2.36	0.001
10	1.16	1.12	2.28	0.0023
Cochran test			Grubbs' test	
C:	0.392		G:	1.931
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				0.012
s^2_{sam}				0.001
sufficient homogeneous if critical value > s^2_{sam}			accepted	

HO_03 (blossom honey):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	3.23	3.17	6.40	0.004
2	3.29	3.34	6.63	0.003
3	3.39	3.26	6.65	0.016
4	3.18	3.33	6.52	0.022
5	3.27	3.29	6.56	0.000
6	3.26	3.19	6.45	0.005
7	3.19	3.12	6.32	0.005
8	3.17	3.15	6.32	0.001
9	3.26	3.17	6.44	0.008
10	3.20	3.07	6.26	0.017
Cochran test			Grubbs' test	
C:	0.288		G:	2.082
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				0.089
s^2_{sam}				0.002
sufficient homogeneous if critical value > s^2_{sam}			accepted	

HO_04 (blossom honey):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	1.93	1.88	3.81	0.002
2	1.91	1.90	3.80	0.0001
3	1.90	1.88	3.78	0.0002
4	1.89	1.84	3.73	0.002
5	1.82	1.81	3.64	0.0001
6	1.81	1.81	3.62	0.00001
7	1.80	1.79	3.60	0.00004
8	1.79	1.83	3.63	0.002
9	1.87	1.78	3.65	0.008
10	1.87	1.79	3.66	0.006
Cochran test			Grubbs' test	
C:	0.372		G:	1.831
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				0.029
s^2_{sam}				0.001
sufficient homogeneous if critical value > s^2_{sam}			accepted	

HO_05 (orange blossom honey):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	2.02	2.00	4.02	0.0002
2	1.99	1.94	3.93	0.002
3	1.98	1.99	3.98	0.0001
4	1.97	1.99	3.96	0.0003
5	2.00	2.00	4.00	0.0001
6	2.03	2.03	4.06	0.00003
7	2.02	2.03	4.05	0.00002
8	2.04	2.06	4.10	0.0003
9	2.02	2.05	4.07	0.001
10	2.04	2.07	4.12	0.001
Cochran test			Grubbs' test	
C:	0.439		G:	2.345
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):			0.033	
s^2_{sam}			0.001	
sufficient homogeneous if critical value > s^2_{sam}			accepted	

Annex C Homogeneity data for retrorsine in tea

PM_01 and PM_05 (melissa tea):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	28.56	29.12	57.68	0.317
2	30.89	29.89	60.78	1.017
3	30.45	28.33	58.78	4.464
4	29.44	26.89	56.33	6.490
5	32.94	32.92	65.86	0.0002
6	27.74	28.38	56.12	0.414
7	27.76	31.23	58.99	12.014
8	31.63	30.85	62.48	0.622
9	29.31	34.42	63.73	26.110
10	32.92	34.17	67.09	1.557
Cochran test			Grubbs' test	
C:	0.492		G:	1.855
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				10.243
s^2_{sam}				2.439
sufficient homogeneous if critical value > s^2_{sam}			accepted	

PM_02 (chamomile tea):

sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	21.01	16.10	37.10	24.117
2	16.56	17.17	33.73	0.372
3	19.17	16.02	35.18	9.936
4	19.65	21.46	41.11	3.258
5	20.12	23.43	43.56	10.939
6	22.01	17.37	39.38	21.553
7	17.03	17.96	34.99	0.853
8	22.62	23.00	45.62	0.146
9	18.09	17.37	35.46	0.520
10	19.38	17.35	36.73	4.094
Cochran test			Grubbs' test	
C:	0.318		G:	1.830
C_{crit}:	0.602		G_{crit}:	2,708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				6.830
s^2_{sam}				2.095
sufficient homogeneous if critical value > s^2_{sam}			accepted	

PM_03 (mixed herbal tea):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	34.98	33.94	68.92	1.086
2	34.27	34.06	68.33	0.046
3	34.47	33.97	68.44	0.243
4	37.33	34.63	71.96	7.248
5	34.09	34.55	68.64	0.213
6	34.94	33.75	68.69	1.427
7	36.38	33.68	70.06	7.342
8	34.02	36.60	70.62	6.667
9	34.11	33.88	67.99	0.052
10	33.57	34.30	67.88	0.536
Cochran- test			Grubbs' test	
C:	0.294		G:	2.740
C_{crit}:	0.602		G_{crit}:	3.001
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				11.044
s^2_{sam}				0.001
sufficient homogeneous if critical value > s^2_{sam}			accepted	

PM_04 (rooibos tea):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	59.53	60.88	120.41	1.839
2	56.17	57.46	113.63	1.665
3	53.80	55.42	109.22	2.612
4	53.10	56.20	109.29	9.596
5	52.29	53.96	106.25	2.813
6	57.05	57.39	114.43	0.115
7	55.81	56.01	111.82	0.038
8	53.93	55.98	109.91	4.187
9	53.22	49.85	103.08	11.378
10	55.41	52.60	108.01	7.923
Cochran test			Grubbs' test	
C:	0.270		G:	2.230
C_{crit}:	0.602		G_{crit}:	2.708
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				27.173
s^2_{sam}				4.726
sufficient homogeneous if critical value > s^2_{sam}			accepted	

PM_06 (black tea):

Sample	a	b	$S_i=(a+b)$	$D_i^2=(a-b)^2$
1	36.47	37.44	73.91	0.941
2	37.35	39.02	76.37	2.793
3	37.87	34.59	72.45	10.761
4	30.40	25.95	56.35	19.840
5	34.73	35.40	70.13	0.442
6	33.06	35.43	68.49	5.600
7*	78.38*	74.78*	153.15	12.967
8	41.64	32.38	74.02	85.849
9	33.29	39.03	72.33	32.960
10	39.45	39.89	79.33	0.195
Cochran test			Grubbs' test	
C:	0.538		G:	2,632
C_{crit}:	0.638		G_{crit}:	2.651
$C < C_{crit}$	no outlier		$G < G_{crit}$	no outlier
Homogeneity				
critical value (h):				19.398
s^2_{sam}				6.183
sufficient homogeneous if critical value > s^2_{sam}			Accepted	
* outlier				

Annex D Submitted data: honey

Recovery sample honey (HO_recovery)																		
[µg/kg] Labcode		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
LC001	I	3.91	4.04	4.76	4.99	9.00	4.00	3.64	3.72	2.36	14.12	3.94	5.88	3.89	4.79	4.32	3.76	4.20	3.24
	II	3.85	4.30	5.19	4.56	8.70	4.10	3.54	3.89	2.61	15.47	4.03	6.56	4.04	5.10	4.27	3.91	4.63	3.37
	s	0.04	0.18	0.30	0.30	0.21	0.07	0.07	0.12	0.18	0.95	0.06	0.48	0.11	0.22	0.04	0.11	0.30	0.09
LC002	I	4.55	3.85	4.52	3.56	7.30	3.75	3.68	4.07	3.96	8.80	3.82	5.52	3.73	5.13	5.10	3.06	1.61	3.70
	II	4.87	4.20	5.08	3.84	7.90	4.09	3.91	4.43	4.09	10.64	4.43	6.03	4.11	5.32	5.50	3.52	2.04	4.08
	s	0.23	0.25	0.40	0.20	0.42	0.24	0.16	0.25	0.09	1.30	0.43	0.36	0.27	0.13	0.28	0.33	0.30	0.27
LC003	I	4.53	5.68	7.29	8.43b	14.50c	6.10	3.94	5.59	5.05	21.26	6.06	9.60b	3.11	7.21	4.83	4.97	7.49	5.45
	II	4.23	5.82	7.02	9.25b	16.20c	6.96	3.77	5.33	5.13	24.67	6.88	10.06b	2.97	7.42	4.50	4.84	7.09	5.43
	s	0.21	0.10	0.19	0.58	1.20	0.61	0.12	0.18	0.06	2.41	0.58	0.33	0.10	0.15	0.23	0.09	0.28	0.01
LC004	I	4.18	3.35	4.06	3.49	7.00	3.46	3.58	3.50	3.40	9.70	4.01	3.14	3.50	3.65	4.37	3.31	2.74	3.29
	II	4.12	3.20	3.93	3.40	6.70	3.25	3.67	3.46	3.50	9.52	3.99	3.76	3.48	3.73	4.18	3.68	2.77	3.40
	s	0.04	0.11	0.09	0.06	0.21	0.15	0.06	0.03	0.07	0.13	0.01	0.44	0.01	0.06	0.13	0.26	0.02	0.08
LC005	I	3.89	3.95	3.90	3.47	6.80	3.31	4.03	3.69	2.52	8.58	3.74	3.90	4.08	3.53	4.96	3.57	2.04	3.43
	II	3.78	3.85	4.05	3.73	7.10	3.33	3.64	3.64	2.68	8.80	4.10	3.58	4.44	3.64	4.47	3.58	1.99	3.28
	s	0.08	0.07	0.11	0.18	0.21	0.01	0.28	0.04	0.11	0.16	0.25	0.23	0.25	0.08	0.35	0.01	0.04	0.11
LC006	I	4.50	3.57	4.82	3.89	7.50	3.58	4.48	4.61	1.24	9.30	4.30	4.40	4.41	5.06	4.93	3.53	2.53	1.78
	II	4.78	3.73	4.92	4.25	8.10	3.80	4.78	4.93	2.08	11.76	4.51	4.34	4.38	5.14	5.19	3.96	2.61	2.42
	s	0.20	0.11	0.07	0.25	0.42	0.16	0.21	0.23	0.59	1.74	0.15	0.04	0.02	0.06	0.18	0.30	0.06	0.45
LC007	I	2.81	2.96	3.90	3.11	5.90	2.81	2.75	2.91	3.71		6.67	2.48	2.54	4.47	3.61	2.80	3.12	5.06
	II	3.11	2.99	3.77	3.08	5.80	2.72	2.96	3.38	3.35		7.45	3.20	3.24	3.26	3.66	3.49	3.66	5.13
	s	0.21	0.02	0.09	0.02	0.07	0.06	0.15	0.33	0.25		0.55	0.51	0.49	0.86	0.04	0.49	0.38	0.05
LC009	I	4.82	4.74	4.85	4.42	8.10	3.64	4.16	4.57	4.24	11.32	3.96	4.77	4.49	4.88	5.01	4.29	3.43	4.11
	II	4.26	4.50	4.68	4.33	8.30	3.93	4.06	4.52	3.83	12.74	4.65	4.13	4.05	5.00	4.98	3.84	3.36	4.01
	s	0.40	0.17	0.12	0.06	0.14	0.21	0.07	0.04	0.29	1.00	0.49	0.45	0.31	0.08	0.02	0.32	0.05	0.07
LC011	I	2.73	2.91	3.55	2.58	5.00	2.45	2.56	3.13	2.17	8.59	2.38	4.19	2.11	3.63	3.03	1.74	1.59	2.18
	II	2.87	2.98	3.54	2.54	5.10	2.54	2.59	3.10	2.24	8.59	2.45	4.16	2.19	3.57	3.05	1.74	1.53	2.21
	s	0.10	0.05	0.01	0.03	0.07	0.06	0.02	0.02	0.05	0.00	0.05	0.02	0.06	0.04	0.01	0.00	0.04	0.02

Recovery sample honey (HO_recovery)																			
[µg/kg] Labcode		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
LC012	I	6.11c	3.43	5.50	4.95	9.80c	4.80	4.46	4.75	5.01	19.12	4.97	3.98	4.37	4.21	5.75	3.97	2.61	3.64
	II	4.89c	2.79	5.92	3.78	7.60c	3.86	4.47	5.79	4.42	15.82	4.55	4.32	5.02	5.38	6.62	3.66	2.90	4.19
	s	0.86	0.45	0.30	0.83	1.56	0.66	0.01	0.74	0.42	2.33	0.30	0.24	0.46	0.83	0.62	0.22	0.21	0.39
LC013	I	5.07	3.57	5.59	3.64	7.30	3.64	4.73	5.12	0.59	9.65	3.22	4.54	3.80	6.63	5.21	2.85	3.42	2.78
	II	4.91	3.15	5.00	3.14	6.60	3.49	4.29	4.41	0.62	7.94	2.66	4.10	3.51	5.17	4.84	2.47	2.58	2.91
	s	0.11	0.30	0.42	0.35	0.49	0.11	0.31	0.50	0.02	1.21	0.40	0.31	0.21	1.03	0.26	0.27	0.59	0.09
LC014	I	3.30	4.85	6.11	3.84	7.60	3.72	3.11	4.01	3.25	14.86	4.61	6.29	3.05	4.24	4.89	4.32	4.14	4.91
	II	3.34	4.66	6.00	4.06	7.90	3.79	3.18	4.06	3.23	15.12	4.78	6.43	3.22	4.37	4.82	4.19	4.13	5.10
	s	0.03	0.13	0.08	0.16	0.21	0.05	0.05	0.04	0.01	0.18	0.12	0.10	0.12	0.09	0.05	0.09	0.01	0.13
LC015	I	~~4.00~~	6.17	5.15	4.75	8.80	4.06	4.30	3.69	2.94	8.89	5.14	3.97	4.55	3.30	6.36	4.35	2.78	4.00
	II	~~3.97~~	6.40	5.17	4.70	8.90	4.19	4.21	3.66	3.03	8.65	5.05	4.13	4.49	3.13	6.66	4.38	2.90	4.39
	s	~~0.02~~	0.16	0.01	0.04	0.07	0.09	0.06	0.02	0.06	0.17	0.06	0.11	0.04	0.12	0.21	0.02	0.08	0.28
LC016	I	5.88	4.39	7.41	5.79	13.70b	7.89	4.99	5.72	4.55	13.38	~~6.16~~	5.37	4.63	6.20	5.77	4.97	4.86	4.36
	II	5.59	4.23	6.76	5.22	13.40b	8.21	5.48	5.19	4.66	13.49	~~4.27~~	5.18	4.50	5.85	6.09	4.54	4.03	3.78
	s	0.21	0.11	0.46	0.40	0.21	0.23	0.35	0.37	0.08	0.08	~~1.34~~	0.13	0.09	0.25	0.23	0.30	0.59	0.41
LC017	I	3.47	2.94	3.73	2.56	6.00	3.41	3.32	3.29	3.77	7.59	4.94	3.19	3.10	3.51	3.82	2.64	2.04	2.96
	II	3.25	3.28	3.84	2.57	5.90	3.36	3.24	3.17	3.93	7.81	5.69	3.18	3.08	3.41	3.95	3.35	2.24	3.23
	s	0.16	0.24	0.08	0.01	0.07	0.04	0.06	0.08	0.11	0.16	0.53	0.01	0.01	0.07	0.09	0.50	0.14	0.19
LC018	I	5.93	5.54	6.95	4.54	8.50c	3.99c	4.76	5.09	5.91	12.01	5.22	6.27	5.01	6.49	6.55	6.50	5.42	4.95
	II	6.23	6.06	6.88	5.85	11.80c	5.98c	4.55	5.32	5.43	15.50	4.74	6.01	5.21	6.61	6.51	5.89	5.29	4.69
	s	0.21	0.37	0.05	0.93	2.33	1.41	0.15	0.16	0.34	2.47	0.34	0.18	0.14	0.08	0.03	0.43	0.09	0.18
LC019	I	4.58	5.05	5.74		6.80	6.80	4.76	5.21	8.01	17.61	5.89	6.17	4.86	5.45	5.74	4.91	2.89	4.90
	II	4.47	4.96	6.13		6.50	6.50	4.50	5.21	7.86	14.81	6.78	6.42	4.82	5.44	5.59	4.48	2.89	5.23
	s	0.08	0.06	0.28		0.21	0.21	0.18	0.00	0.11	1.98	0.63	0.18	0.03	0.01	0.11	0.30	0.00	0.23
LC020	I	4.19	3.75	4.30	3.53	7.10	3.61	3.95	3.71	3.51	8.81	4.71	3.88	3.99	4.19	4.95	2.68	0.87	3.39
	II	4.10	3.52	4.08	3.71	7.40	3.68	3.91	3.69	3.53	9.16	4.55	3.75	3.83	4.07	4.91	2.56	0.75	3.19
	s	0.06	0.16	0.16	0.13	0.21	0.05	0.03	0.01	0.01	0.25	0.11	0.09	0.11	0.08	0.03	0.08	0.08	0.14
LC023	I	3.98	4.14	5.21	3.86	7.60	3.74	4.07	4.25	4.97	10.05	3.84	5.11	3.70	4.66	4.51	3.23	3.48	3.70
	II	3.84	3.99	5.44	3.84	7.70	3.89	3.97	4.17	5.30	11.07	3.98	5.31	3.44	5.14	4.37	3.17	3.63	3.65
	s	0.10	0.11	0.16	0.01	0.07	0.11	0.07	0.06	0.23	0.72	0.10	0.14	0.18	0.34	0.10	0.04	0.11	0.04

Recovery sample honey (HO_recovery)																		
[µg/kg] Labcode		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
LC118	I	5.33	4.71	6.14		10.01	10.01	5.05	5.82	3.98	13.79	5.51	6.18	5.37	4.89	5.55	5.56	4.31	5.06
	II	5.55	4.82	6.12		10.18	10.18	4.94	5.43	4.17	14.61	5.15	6.05	5.76	4.73	5.77	5.29	4.00	4.99
	s	0.16	0.08	0.01		0.12	0.12	0.08	0.28	0.13	0.58	0.25	0.09	0.28	0.11	0.16	0.19	0.22	0.05

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Honey sample HO_01						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC001	I	4.55	2.64	1.56	1.24	
	II	4.45	2.66	1.75	1.30	
	s	0.07	0.01	0.13	0.04	
LC002	I	6.06	2.83	2.16	1.22	0.49
	II	6.23	3.40	2.38	1.44	0.53
	s	0.12	0.40	0.16	0.16	0.03
LC003	I	3.02	3.61	2.19	0.79	
	II	2.97	3.94	1.64	0.81	
	s	0.04	0.23	0.39	0.01	
LC004	I	5.12	10.97b	2.03	0.99	
	II	5.25	10.81b	2.05	0.92	
	s	0.09	0.11	0.01	0.05	
LC005	I	3.67	3.11	1.38	1.22	
	II	4.01	3.51	1.55	1.10	
	s	0.24	0.28	0.12	0.08	
LC006	I	4.07	1.88	1.48	0.81	0.52
	II	4.16	1.91	1.54	0.81	0.47
	s	0.06	0.02	0.04	0.00	0.04
LC007	I	3.59	1.57	0.95	1.04	0.56
	II	3.72	1.22	0.80	1.24	0.64
	s	0.09	0.25	0.11	0.14	0.06
LC009	I	4.27	2.78	2.00	0.42	
	II	4.07	2.86	1.93	0.45	
	s	0.14	0.06	0.05	0.02	
LC011	I	5.29	2.28	1.91	1.23	0.54
	II	5.21	2.27	1.89	1.16	0.55
	s	0.06	0.01	0.01	0.05	0.01
LC012	I	5.75	4.82		1.21	0.67
	II	5.27	4.56		1.34	0.73
	s	0.34	0.18		0.09	0.04
LC013	I					
	II					
	s					
LC014	I	3.78	1.58	2.54	0.85	0.61
	II	3.90	1.69	2.49	1.03	0.66
	s	0.08	0.08	0.04	0.13	0.04
LC015	I	~~8.32~~	1.69	2.45	1.36	0.73
	II	~~7.30~~	1.75	2.65	1.52	0.88
	s	~~0.72~~	0.04	0.14	0.11	0.11
LC016	I	5.76	0.72	~~2.28~~	0.97	
	II	5.59	0.45	~~1.66~~	0.83	
	s	0.12	0.19	~~0.44~~	0.10	
LC017	I	3.76	4.63	1.23	0.92	
	II	3.75	4.62	1.69	0.72	
	s	0.01	0.01	0.33	0.14	
LC018	I	3.69	2.61	1.46	0.69	0.53
	II	3.61	1.80	1.34	0.72	0.43
	s	0.06	0.57	0.08	0.02	0.07

Honey sample HO_01						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC019	I	4.11	5.11	2.37	1.06	0.52
	II	4.02	5.08	2.08	0.82	
	s	0.06	0.02	0.21	0.17	
LC020	I	4.57	1.64	2.27	1.42	0.48
	II	4.55	1.56	2.30	1.38	0.39
	s	0.01	0.06	0.02	0.03	0.06
LC023	I	4.11	6.92	1.72	1.08	0.61
	II	3.27	6.69	1.71	0.91	0.48
	s	0.59	0.16	0.01	0.12	0.09
LC118	I	4.41	2.09	1.68	0.69	
	II	5.24	2.23	1.58	0.80	
	s	0.59	0.10	0.07	0.08	
crossed out value: conspicuous and removed value during first Mandel h and k statistic b: Grubb outlier c: Cochran outlier I: first injection value II: second injection value s: standard deviation						

Honey sample HO_02						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC001	I	1.14	3.98	3.39	2.71	1.11
	II	1.10	3.75	3.18	2.94	1.17
	s	0.03	0.16	0.15	0.16	0.04
LC002	I	1.39	1.07c	3.36	2.43	0.94
	II	1.46	4.69c	3.97	2.82	1.17
	s	0.05	2.56	0.43	0.28	0.16
LC003	I	0.93	6.87	4.12	1.53	1.05
	II	0.87	8.44	3.27	1.51	1.23
	s	0.04	1.11	0.60	0.01	0.13
LC004	I	1.34	6.87	3.39	2.06	0.88
	II	1.33	7.01	3.51	2.06	0.88
	s	0.01	0.10	0.08	0.00	0.00
LC005	I	0.95	3.80	2.58	2.24	0.59
	II	1.04	4.22	2.77	2.63	0.75
	s	0.06	0.30	0.13	0.28	0.11
LC006	I	1.19	3.17	3.36	1.84	1.00
	II	1.18	3.53	3.71	1.89	1.01
	s	0.01	0.25	0.25	0.04	0.01
LC007	I	1.00	3.21	1.17	2.01	1.58
	II	1.09	2.25	1.20	1.64	1.19
	s	0.06	0.68	0.02	0.26	0.28
LC009	I	1.20	3.64	3.57	1.83	1.46
	II	1.26	3.49	3.43	1.78	1.32
	s	0.04	0.11	0.10	0.04	0.10
LC011	I	1.47	2.97	3.59	2.24	0.99
	II	1.44	2.98	3.61	2.29	0.99

Honey sample HO_02						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
	s	0.02	0.01	0.01	0.04	0.00
LC012	I	1.62	7.33		3.46	2.15
	II	1.45	6.40		3.61	1.92
	s	0.12	0.66		0.11	0.16
LC013	I	0.95	3.92	2.47	1.75	1.10
	II	0.93	3.87	2.57	1.64	1.13
	s	0.01	0.04	0.07	0.08	0.02
LC014	I	0.89	2.55	4.03	1.65	1.20
	II	0.95	2.67	4.07	1.81	1.37
	s	0.04	0.08	0.03	0.11	0.12
LC015	I	~~1.82~~	2.72	4.50	2.53	1.76
	II	~~1.77~~	2.52	4.87	2.74	1.92
	s	~~0.04~~	0.14	0.26	0.15	0.11
LC016	I	1.39	0.97	~~3.24~~	2.24	0.85
	II	1.42	0.97	~~2.79~~	2.18	1.05
	s	0.02	0.00	~~0.32~~	0.04	0.14
LC017	I	0.84	5.16	2.50	1.83	0.82
	II	0.84	5.19	2.69	1.75	0.72
	s	0.00	0.02	0.13	0.06	0.07
LC018	I	0.99	3.44	3.10	1.38	1.07
	II	0.94	2.59	2.50	1.52	0.92
	s	0.04	0.60	0.42	0.10	0.11
LC019	I	0.60	5.30	1.93	1.19	0.81
	II	0.60	6.05	1.97	1.24	0.66
	s	0.00	0.53	0.03	0.04	0.11
LC020	I	1.19	1.97	3.83	2.63	0.93
	II	1.21	1.95	4.11	2.78	0.92
	s	0.01	0.01	0.20	0.11	0.01
LC023	I	1.29c	8.70	2.85	2.05	1.12
	II	0.89c	9.41	2.87	1.74	0.94
	s	0.28	0.50	0.01	0.22	0.13
LC118	I	1.01	2.89	2.99	1.47	0.99
	II	1.05	3.17	3.08	1.55	1.03
	s	0.03	0.20	0.06	0.06	0.03

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Honey sample HO_03						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC001	I	3.38	4.32	0.86	2.60	1.07
	II	3.36	4.70	0.99	2.82	1.26
	s	0.01	0.27	0.09	0.16	0.13
LC002	I	4.27	1.94	0.88	1.80	0.56
	II	4.50	3.48	1.10	2.13	0.63
	s	0.16	1.09	0.16	0.23	0.05
LC003	I	2.48	3.99	0.80	1.25	0.63
	II	2.49	3.56	0.79	1.21	0.62
	s	0.01	0.30	0.01	0.03	0.01
LC004	I	3.55	6.09	0.99	1.49	0.92
	II	3.57	5.94	1.01	1.38	0.98
	s	0.01	0.11	0.01	0.08	0.04
LC005	I	2.40	4.13	0.61	1.83	0.52
	II	2.64	4.33	0.69	2.13	0.65
	s	0.17	0.14	0.06	0.21	0.09
LC006	I	3.02	4.42	0.82	1.24	0.98
	II	2.81	4.68	0.76	1.17	0.91
	s	0.15	0.18	0.04	0.05	0.05
LC007	I	2.45	3.94	1.65	1.12c	1.58c
	II	2.73	3.37	1.07	2.12c	2.34c
	s	0.20	0.40	0.41	0.71	0.54
LC009	I	3.36	5.22	1.32	0.91	1.39
	II	2.93	5.27	1.34	0.90	1.33
	s	0.30	0.04	0.01	0.01	0.04
LC011	I	3.54	3.27	0.94	1.93	0.82
	II	3.60	3.32	0.92	1.96	0.82
	s	0.04	0.04	0.01	0.02	0.00
LC012	I	3.47	5.56		1.95	1.71
	II	3.13	5.15		2.08	1.56
	s	0.24	0.29		0.09	0.11
LC013	I	2.62	2.79		1.42	1.40
	II	2.74	2.86	0.54	1.45	1.48
	s	0.08	0.05		0.02	0.06
LC014	I	2.47	2.86	0.86	1.36	1.11
	II	2.53	3.02	0.98	1.47	1.22
	s	0.04	0.11	0.08	0.08	0.08
LC015	I	~~5.41~~	4.88	0.92	1.59	1.51
	II	~~5.73~~	5.09	1.06	1.91	1.84
	s	~~0.23~~	0.15	0.10	0.23	0.23
LC016	I	4.24	2.14		1.85	1.32
	II	4.19	1.40		1.17	1.27
	s	0.04	0.52		0.48	0.04
LC017	I	2.62	4.48	1.09	1.37	1.11
	II	2.32	4.35	0.81	1.38	0.98
	s	0.21	0.09	0.20	0.01	0.09
LC018	I	2.93	5.65	0.86	1.42	1.06
	II	2.64	4.17	0.52	1.37	1.00
	s	0.21	1.05	0.24	0.04	0.04

Honey sample HO_03						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC019	I	2.01	3.78		1.28	0.76
	II	2.05	4.32	0.54	1.27	0.85
	s	0.03	0.38		0.01	0.06
LC020	I		3.79			
	II	3.17	3.86	1.02	1.70	1.04
	s		0.05			
LC023	I	2.92	4.67	0.86	1.51	1.07
	II	2.58	4.58	0.67	1.28	0.94
	s	0.24	0.06	0.13	0.16	0.09
LC118	I	2.35	4.75	0.58	1.33	1.06
	II	2.53	4.87	0.58	1.42	1.01
	s	0.13	0.08	0.00	0.06	0.04
crossed out value: conspicuous and removed value during first Mandel h and k statistic b: Grubb outlier c: Cochran outlier I: first injection value II: second injection value s: standard deviation						

Honey sample HO_04						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC001	I	1.97	2.74	3.09	7.76	2.93
	II	1.97	2.76	3.15	8.59	3.13
	s	0.00	0.01	0.04	0.59	0.14
LC002	I	2.89	1.17	3.51	8.05	3.12
	II	3.12	1.32	4.27	8.20	3.52
	s	0.16	0.11	0.54	0.11	0.28
LC003	I	1.65	3.99	4.30	4.96	2.94
	II	1.24	4.08	3.57	3.57	2.31
	s	0.29	0.06	0.52	0.98	0.45
LC004	I	2.07	2.93	2.81	5.25	2.06
	II	2.07	3.02	3.01	5.23	2.14
	s	0.00	0.06	0.14	0.01	0.06
LC005	I	1.53	2.06	2.22	6.07	1.85
	II	1.68	2.10	2.50	6.35	2.09
	s	0.11	0.03	0.20	0.20	0.17
LC006	I	1.73	2.02	2.44	4.08	2.12
	II	1.86	2.24	2.67	4.54	2.34
	s	0.09	0.16	0.16	0.33	0.16
LC007	I	1.40	1.02	2.24	3.78	2.39
	II	1.46	1.11	2.09	3.79	3.25
	s	0.04	0.06	0.11	0.01	0.61
LC009	I	~~2.03~~	~~5.79~~	~~8.18~~	~~14.34~~	~~11.64~~
	II	~~2.02~~	~~5.77~~	~~8.50~~	~~12.92~~	~~10.53~~
	s	~~0.01~~	~~0.01~~	~~0.23~~	~~1.00~~	~~0.78~~

Honey sample HO_04						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC011	I	2.01	1.88	2.90	5.79	2.22
	II	2.10	1.88	2.86	5.81	2.29
	s	0.06	0.00	0.03	0.01	0.05
LC012	I	2.59	3.71c		8.26	5.05
	II	2.04	2.58c		8.88	4.68
	s	0.39	0.80		0.44	0.26
LC013	I					
	II					
	s					
LC014	I	1.38	1.61	3.45	3.97	3.14
	II	1.49	1.71	3.58	4.17	3.08
	s	0.08	0.07	0.09	0.14	0.04
LC015	I	~~3.09~~	2.04	3.99	6.66	4.52
	II	~~2.96~~	2.05	3.84	7.20	4.35
	s	~~0.09~~	0.01	0.11	0.38	0.12
LC016	I	2.68	2.56	4.82	7.20	3.10
	II	2.69	2.47	3.67	5.96	3.72
	s	0.01	0.06	0.81	0.88	0.44
LC017	I	1.32	2.35	3.45	3.50	2.07
	II	1.17	2.00	3.18	3.62	1.81
	s	0.11	0.25	0.19	0.08	0.18
LC018	I	1.71	2.31	2.82	4.24	2.55
	II	1.59	1.78	2.03	3.69	2.63
	s	0.08	0.37	0.56	0.39	0.06
LC019	I	0.74	1.75	1.78	2.69	1.35
	II	0.65	2.15	1.61	3.12	1.42
	s	0.06	0.28	0.12	0.30	0.05
LC020	I	1.74	1.76	3.00	6.49	2.13
	II	1.70	1.70	3.22	6.73	2.17
	s	0.03	0.04	0.16	0.17	0.03
LC023	I	1.71	3.80	2.41	4.60	2.30
	II	1.42	3.55	2.42	4.29	2.10
	s	0.21	0.18	0.01	0.22	0.14
LC118	I	2.02	2.66	3.07	4.09	2.83
	II	1.97	2.51	2.94	4.02	2.76
	s	0.04	0.11	0.09	0.05	0.05

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Honey sample HO_05						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC001	I	1.81	3.80	7.08	23.31	7.89
	II	1.87	3.74	8.58	25.57	9.01
	s	0.04	0.04	1.06	1.60	0.79
LC002	I	2.06	1.67	8.41	21.07	7.06
	II	2.20	3.49	10.47	20.76	8.07
	s	0.10	1.29	1.46	0.22	0.71
LC003	I	1.36	7.20	8.71	11.01	7.80
	II	1.26	7.80	8.32	10.99	7.10
	s	0.07	0.42	0.28	0.01	0.49
LC004	I	2.14	11.24	8.82	14.86	6.88
	II	2.07	11.08	9.13	15.06	6.82
	s	0.05	0.11	0.22	0.14	0.04
LC005	I	1.68	4.04	6.15	20.07	5.29
	II	1.76	3.91	6.72	20.46	5.16
	s	0.06	0.09	0.40	0.28	0.09
LC006	I	1.76	3.18	7.57	13.45	7.21
	II	1.78	3.66	8.64	14.33	7.42
	s	0.01	0.34	0.76	0.62	0.15
LC007	I	1.58	1.60	1.65	18.75	11.88
	II	1.80	1.24		17.63	10.39
	s	0.16	0.25		0.79	1.05
LC009	I	1.74	2.17	2.82	4.54	2.91
	II	1.77	2.17	2.85	4.09	2.70
	s	0.02	0.00	0.02	0.32	0.15
LC011	I	2.21	3.78	7.72	15.78	5.03
	II	2.24	3.83	7.66	15.61	4.87
	s	0.02	0.04	0.04	0.12	0.11
LC012	I	2.55	5.12		19.12	13.12
	II	2.03	4.14		20.29	11.98
	s	0.37	0.69		0.83	0.81
LC013	I					
	II					
	s					
LC014	I	1.74	3.92	12.52	16.57	12.98
	II	1.81	4.10	12.63	17.37	12.78
	s	0.05	0.13	0.08	0.57	0.14
LC015	I	~~3.58~~	5.02	10.60	19.62	14.92
	II	~~3.70~~	5.59	10.39	21.79	14.59
	s	~~0.08~~	0.40	0.15	1.53	0.23
LC016	I	2.65	1.08	~~11.44~~	24.11	9.89
	II	2.94	1.18	~~13.16~~	19.73	10.58
	s	0.21	0.07	~~1.22~~	3.10	0.49
LC017	I	1.70	5.26	11.08	15.61	7.87
	II	1.55	4.22	12.06	15.00	8.73
	s	0.11	0.74	0.69	0.43	0.61
LC018	I	2.06	7.10	11.74c	16.59	10.08
	II	1.93	5.42	6.72c	15.92	9.67
	s	0.09	1.19	3.55	0.47	0.29

Honey sample HO_05						
[µg/kg] Labcode		Em	Im/La	Re	Sc	Sp
LC019	I	1.51	4.57	8.21	16.33	9.35
	II	1.63	5.12	11.36	19.47	10.11
	s	0.08	0.39	2.23	2.22	0.54
LC020	I	2.09	3.66	10.15	18.51	7.95
	II	2.03	3.83	10.74	19.34	8.28
	s	0.04	0.12	0.42	0.59	0.23
LC023	I	2.06	7.20	7.67	15.24	7.28
	II	1.81	7.25	7.93	14.30	7.12
	s	0.18	0.04	0.18	0.66	0.11
LC118	I	1.47	5.75	7.24	14.97	9.15
	II	1.61	5.84	6.81	14.25	8.79
	s	0.10	0.06	0.30	0.51	0.25
crossed out value: conspicuous and removed value during first Mandel h and k statistic b: Grubb outlier c: Cochran outlier I: first injection value II: second injection value s: standard deviation						

Honey sample HO_06						
[µg/kg]		Em	Im/La	Re	Sc	Sp
Labcode						
LC001	I	0.97	4.43	2.95	2.33	0.99
	II	0.98	4.19	2.95	2.70	1.05
	s	0.01	0.17	0.00	0.26	0.04
LC002	I	1.39	0.58	2.85	1.95	0.86
	II	1.43	3.39	3.38	2.41	1.04
	s	0.03	1.99	0.37	0.33	0.13
LC003	I	1.05	7.90	3.81	2.19	1.31
	II	1.02	9.25	4.91	2.07	1.14
	s	0.02	0.95	0.78	0.08	0.12
LC004	I	1.32	7.26	3.23	1.98	0.79
	II	1.28	8.86	3.39	1.99	0.89
	s	0.03	1.13	0.11	0.01	0.07
LC005	I	0.95	3.13	2.53	2.59	0.68
	II	0.99	3.47	2.86	2.56	0.78
	s	0.03	0.24	0.23	0.02	0.07
LC006	I	1.08	2.88	2.78	1.75	0.99
	II	1.10	3.27	3.27	1.71	1.02
	s	0.01	0.28	0.35	0.03	0.02
LC007	I	1.01	1.62	3.43	1.93	0.96c
	II	0.90	1.33	2.75	1.98	1.55c
	s	0.08	0.21	0.48	0.04	0.42
LC009	I	1.08	3.93	3.46	1.99	1.31
	II	1.13	3.89	2.86	1.84	1.26
	s	0.04	0.03	0.42	0.11	0.04
LC011	I	1.47	2.94	3.62	2.41	1.07
	II	1.51	2.96	3.63	2.46	1.04
	s	0.03	0.01	0.01	0.04	0.02

Honey sample HO_06						
[µg/kg]		Em	Im/La	Re	Sc	Sp
Labcode						
LC012	I	1.71c	7.65		3.03	1.87
	II	1.32c	5.55		3.39	1.77
	s	0.28	1.48		0.25	0.07
LC013	I	1.18	2.27	3.26	2.11	1.34
	II	1.10	2.11	2.99	1.99	1.25
	s	0.06	0.11	0.19	0.08	0.06
LC014	I	0.85	1.79	3.87	1.47	1.11
	II	0.90	1.86	3.82	1.69	1.16
	s	0.04	0.05	0.04	0.16	0.04
LC015	I	~~1.93~~	1.48	4.45	2.72	1.82
	II	~~1.92~~	2.51	4.58	2.80	1.89
	s	~~0.01~~	0.73	0.09	0.06	0.05
LC016	I	~~1.86~~	~~6.01~~	~~5.99~~	~~4.85~~	~~3.63~~
	II	~~1.71~~	~~7.15~~	~~4.39~~	~~3.51~~	~~3.40~~
	s	~~0.11~~	~~0.81~~	~~1.13~~	~~0.95~~	~~0.16~~
LC017	I	0.85	5.14	2.88	1.80	0.79
	II	0.80	5.14	2.90	1.77	0.62
	s	0.04	0.00	0.01	0.02	0.12
LC018	I	1.14	3.65	3.75	1.60	1.09
	II	1.12	2.83	2.78	1.91	1.12
	s	0.01	0.58	0.69	0.22	0.02
LC019	I		~~4.89~~	~~1.10~~	~~0.41~~	
	II		~~5.49~~	~~0.74~~	~~0.48~~	
	s		~~0.42~~	~~0.25~~	~~0.05~~	
LC020	I	1.20	1.97	3.77	2.67	0.92
	II	1.16	1.98	4.14	2.59	0.87
	s	0.03	0.01	0.26	0.06	0.04
LC023	I	1.26	8.70	2.62	2.12	1.18
	II	0.97	8.85	2.94	1.80	0.98
	s	0.21	0.11	0.23	0.23	0.14
LC118	I	0.96	3.05	3.21	1.73	1.11
	II	1.04	3.43	3.01	1.86	1.22
	s	0.06	0.27	0.14	0.09	0.08

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Annex E Submitted data tea

Recovery sample tea (PM_recovery)																			
[µg/kg]		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
Labcode																			
LC001	I	~~16.8~~	~~33.9~~	~~65.1~~	~~9.3~~	~~15.6~~	~~6.3~~	~~41.2~~	~~47.4~~	~~13.9~~	~~69.8~~		~~70.0~~		~~66.3~~	~~97.9~~		~~47.8~~	~~24.5~~
	II	~~16.2~~	~~31.0~~	~~66.0~~	~~4.0~~	~~11.6~~	~~7.6~~	~~39.5~~	~~45.5~~	~~12.1~~	~~66.8~~		~~67.7~~		~~63.0~~	~~100.2~~		~~43.6~~	~~23.8~~
	s	~~0.4~~	~~2.1~~	~~0.6~~	~~3.7~~	~~2.8~~	~~0.9~~	~~1.2~~	~~1.3~~	~~1.3~~	~~2.1~~		~~1.6~~		~~2.3~~	~~1.6~~		~~3.0~~	~~0.5~~
LC002	I	105.8	115.0	122.2	107.5	222.6	115.1	~~83.0~~	85.7	115.5	100.8	90.3	106.9	90.8	101.4	102.2	112.0	102.5	89.4
	II	107.6	114.9	124.3	105.2	222.4	117.2	~~89.1~~	85.9	115.0	101.2	90.8	105.7	85.9	101.0	102.1	109.0	103.9	90.8
	s	1.3	0.1	1.5	1.6	0.1	1.5	~~4.3~~	0.1	0.4	0.3	0.4	0.8	3.5	0.3	0.1	2.1	1.0	1.0
LC003	I	218.7	91.9	214.3	79.8	211.8	132.0	105.1	99.3	120.5	107.9	130.4	149.5	112.7	121.2	~~311.6~~	121.5	99.8	149.7
	II	220.2	88.3	188.6	93.4	211.7	118.3	111.1	110.4	117.6	118.7	154.9	155.5	111.6	133.9	~~297.6~~	124.1	96.7	150.5
	s	1.1	2.5	18.2	9.7	0.0	9.7	4.2	7.9	2.1	7.7	17.3	4.2	0.8	9.0	~~9.9~~	1.9	2.2	0.5
LC004	I	138.8	94.8	111.8	123.0	257.5	134.5	100.3	103.1	101.9	115.7	93.3	99.0	78.6	95.5	104.6	82.0	79.5	96.3
	II	134.3	88.4	105.5	109.7	234.8	125.1	96.2	95.9	96.6	109.3	85.0	85.1	73.7	85.8	97.6	78.6	72.7	88.5
	s	3.2	4.5	4.5	9.4	16.1	6.7	2.9	5.1	3.8	4.5	5.8	9.8	3.5	6.8	4.9	2.3	4.8	5.5
LC005	I	209.3	139.1	178.4	164.9	338.5	173.6	161.5	183.6	128.0	149.9	105.5	148.1	112.8	169.3	189.7	66.5	155.0	123.2
	II	203.0	148.6	186.6	166.2	334.8	168.6	170.6	176.3	126.8	150.7	107.7	146.9	110.6	170.8	217.1	76.9	138.4	119.2
	s	4.5	6.7	5.8	0.9	2.6	3.5	6.5	5.1	0.9	0.6	1.6	0.9	1.5	1.1	19.3	7.4	11.7	2.8
LC006	I	165.7	125.7	132.1	166.3	342.6	176.3	101.6	103.8	181.2	166.4	130.7	121.9	100.5	101.9	93.3	114.2	108.2	132.4
	II	160.1	122.9	128.1	142.1	303.5	161.4	109.8	111.6	163.1	141.3	131.3	111.0	97.8	111.2	98.5	120.4	111.0	123.0
	s	4.0	2.0	2.8	17.1	27.6	10.6	5.7	5.5	12.8	17.7	0.4	7.7	1.9	6.5	3.6	4.4	2.0	6.6
LC007	I	107.4	162.1	142.3	132.6	272.7	140.0	101.5	105.1	111.0	129.8	128.0	129.9	113.0	111.3	109.0	151.9	94.3	136.5
	II	116.8	156.6	140.1	147.7	287.4	139.7	131.0	97.3	115.1	150.4	125.6	138.2	141.1	122.2	110.9	134.5	122.2	134.0
	s	6.6	3.9	1.5	10.7	10.4	0.2	20.9	5.6	2.9	14.5	1.7	5.9	19.9	7.7	1.4	12.3	19.7	1.8
LC009	I	118.4	133.4	132.4	130.7	255.6	125.0	125.4	151.0	114.3	81.2	118.7	88.5	100.7	90.6	102.2	400.9b	102.9	128.4
	II	113.6	128.5	129.3	126.9	245.6	118.7	125.3	133.5	116.8	73.7	110.8	87.2	89.2	86.2	102.5	401.9b		120.9
	s	3.4	3.5	2.2	2.7	7.1	4.4	0.1	12.4	1.8	5.3	5.6	0.9	8.1	3.1	0.2	0.8		5.4
LC011	I	138.7	158.9	153.4	151.1	296.2	145.2	105.2	142.1	130.1	128.8	81.1	202.0	61.0	241.4b	125.0	87.4	103.3	113.6
	II	139.5	160.5	155.1	151.1	295.3	144.2	108.5	140.3	126.6	124.6	77.5	196.9	57.2	216.7b	128.2	89.3	109.3	113.0
	s	0.6	1.1	1.2	0.0	0.6	0.7	2.3	1.3	2.5	2.9	2.5	3.6	2.7	17.5	2.3	1.3	4.3	0.4

Recovery sample tea (PM_recovery)																			
[µg/kg]		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
Labcode																			
LC012	I	~~167.8~~	~~199.4~~	~~135.4~~	~~164.5~~	~~347.2~~	~~182.7~~	~~172.2~~	~~123.7~~	~~142.0~~	~~122.7~~	~~156.0~~	~~146.1~~	~~156.6~~	~~140.5~~	~~159.8~~	~~137.5~~	~~149.4~~	~~124.2~~
	II	~~96.0~~	~~130.7~~	~~115.4~~	~~105.9~~	~~220.4~~	~~114.5~~	~~157.8~~	~~109.9~~	~~79.5~~	~~71.0~~	~~82.2~~	~~94.9~~	~~112.8~~	~~118.9~~	~~115.7~~	~~69.3~~	~~89.9~~	~~102.3~~
	s	~~50.8~~	~~48.5~~	~~14.2~~	~~41.4~~	~~89.7~~	~~48.2~~	~~10.2~~	~~9.8~~	~~44.2~~	~~36.6~~	~~52.1~~	~~36.2~~	~~31.0~~	~~15.2~~	~~31.2~~	~~48.2~~	~~42.1~~	~~15.5~~
LC013	I	116.2	123.4	130.3	128.9	250.1	121.2	98.3	119.7	119.5	126.7	85.4		65.8	108.1	107.5	144.4	~~73.0~~	108.7
	II	111.6	126.5	151.2	137.5	263.7	126.2	93.0	117.0	108.9	142.7	85.4		87.7	105.4	110.4	160.4	~~30.9~~	105.9
	s	3.2	2.2	14.7	6.0	9.6	3.5	3.7	1.9	7.5	11.3	0.0		15.5	1.9	2.1	11.3	~~29.8~~	1.9
LC014	I	131.0	132.9	153.2	153.9	297.0	143.1	106.2	87.4	93.6	135.9	93.9	119.2	104.0	114.6	139.4	178.2	91.3	121.2
	II	133.5	138.7	158.9	153.4	297.9	144.5	117.4	100.3	98.4	145.6	101.8	128.1	108.5	119.8	147.2	194.5	101.7	131.7
	s	1.7	4.1	4.0	0.4	0.6	0.9	8.0	9.1	3.4	6.9	5.6	6.3	3.2	3.7	5.5	11.5	7.3	7.4
LC015	I	181.7	135.9	169.8	123.0	246.5	123.6	81.9	105.3	86.4	85.5	77.9	106.1	104.4	122.1	115.5	129.1	91.8	105.1
	II	218.2	157.6	206.8	148.3	295.4	147.1	91.3	125.6	108.0	99.2	91.1	124.7	115.9	164.0	129.4	159.3	111.6	121.3
	s	25.8	15.4	26.1	17.9	34.6	16.7	6.7	14.4	15.3	9.7	9.3	13.1	8.1	29.6	9.9	21.3	14.0	11.5
LC016	I	153.0	189.5	159.2	205.1	407.1	202.0	136.4	142.4	161.2	109.8	123.1	152.6	144.3	142.3	145.1	106.8	132.5	151.6
	II	162.1	172.8	158.8	195.1	387.0	191.9	138.9	149.1	141.9	114.1	136.0	153.3	138.3	156.5	141.5	96.4	141.5	137.4
	s	6.4	11.8	0.3	7.1	14.2	7.1	1.7	4.7	13.6	3.1	9.1	0.5	4.2	10.1	2.6	7.4	6.3	10.0
LC017	I																		
	II																		
	s																		
LC018	I	103.6	106.9	117.9	117.8	230.2	112.3	93.2	92.1	100.2	106.6	90.3	97.3	112.7	101.4	109.9	89.5	101.1	96.5
	II	89.2	90.7	101.9	99.9	196.9	97.0	76.7	76.2	86.4	91.8	75.7	84.1	95.8	88.7	103.1	78.6	84.7	83.2
	s	10.2	11.5	11.3	12.7	23.5	10.8	11.7	11.2	9.8	10.5	10.3	9.3	12.0	9.0	4.8	7.7	11.6	9.4
LC019	I	118.0	158.0	184.3		132.8	132.8	122.9	111.5	111.6	96.2	95.7	177.0	117.6	117.3	127.1	131.6	128.9	166.0
	II	121.5	176.6	184.7		168.5	168.5	128.1	124.4	134.2	114.6	94.5	170.1	123.6	116.7	136.4	179.0	120.3	176.5
	s	2.5	13.2	0.3		25.2	25.2	3.6	9.1	15.9	13.1	0.8	4.9	4.2	0.4	6.6	33.5	6.1	7.4
LC020	I	155.3	160.3	176.8	176.8	345.3	168.5	139.7	138.2	150.4	159.9	135.4	145.9	169.0	152.0	164.8	134.2	151.7	144.7
	II	133.8	136.0	152.9	149.9	295.4	145.5	115.1	114.3	129.7	137.7	113.5	126.1	143.7	133.1	154.7	118.0	127.1	124.8
	s	15.2	17.2	16.9	19.0	35.3	16.3	17.4	16.9	14.6	15.7	15.5	14.0	17.9	13.4	7.2	11.5	17.4	14.1
LC023	I	148.7	67.0	118.3	112.8	239.8	127.0	104.0	119.1	124.3	131.6	113.4	107.2	103.1	99.2	112.9	150.2	91.7	112.4
	II	148.9	68.6	115.3	108.0	238.3	130.3	105.6	119.9	126.0	128.0	109.2	107.1	103.0	94.6	113.1	151.2	90.5	116.2
	s	0.1	1.2	2.1	3.4	1.1	2.3	1.1	0.6	1.2	2.5	3.0	0.0	0.1	3.2	0.1	0.7	0.8	2.6

Recovery sample tea (PM_recovery)																			
[µg/kg]		Em	Hn	HnN	Im	Im/La	La	Lc	LcN	Mc	McN	Re	ReN	Sc	ScN	Sk	Sp	SpN	Td
Labcode																			
LC025	I	134.3	176.5	206.6	157.9	331.7	173.8	122.5	113.7	153.8	105.7	59.3	101.1	115.9	128.1	123.8	103.7	136.7	92.9
		134.9	178.4	203.3	149.6	317.5	167.9	118.7	110.8	151.0	105.5	51.3	103.1	129.5	116.4	121.4	99.3	134.0	97.4
	s	0.4	1.4	2.4	5.9	10.0	4.2	2.7	2.0	2.0	0.1	5.7	1.5	9.6	8.3	1.7	3.1	1.9	3.2
LC118	I	106.8	100.6	116.1		230.1	230.1	89.5	98.1	103.4	130.1	93.4	~~93.4~~	90.2	85.6	102.3	98.1	98.9	96.7
	II	84.8	91.0	90.2		172.6	172.6	86.1	90.6	67.9	116.3	80.8	~~57.7~~	73.4	74.0	76.9	84.5	75.4	80.3
	s	15.5	6.8	18.3		40.7	40.6	2.4	5.3	25.1	9.8	8.9	~~25.2~~	11.9	8.2	17.9	9.6	16.6	11.6

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Sample: PM_01											
[µg/kg] Labcode		Hn	HnN	Im/La	Lc	LcN	Re	ReN	Sc	ScN	SpN
LC001	I		44.9			13.5		47.6		67.6	
	II		40.2			11.2		44.9		68.6	
	s		3.3			1.6		2.0		0.7	
LC002	I	40.3	152.9	17.0	~~27.1~~	55.8	37.0	131.0	59.9	143.1	97.5
	II	39.7	154.4	16.3	~~28.2~~	56.0	36.3	127.7	53.0	138.9	97.6
	s	0.5	1.0	0.5	~~0.8~~	0.1	0.4	2.3	4.9	3.0	0.1
LC003	I	43.0	173.6	8.7	8.9	35.1	40.7	127.4	28.9	119.4	80.8
	II	44.8	163.7	11.2	9.2	30.4	37.3	126.3	25.6	130.3	77.1
	s	1.2	7.0	1.8	0.2	3.4	2.4	0.8	2.4	7.8	2.7
LC004	I	42.7	182.0	89.8b	9.8	75.4	12.7	60.7	11.4	88.3	75.6
	II	43.6	180.5	88.8b	9.7	77.1	13.9	54.6	11.6	76.6	79.7
	s	0.6	1.0	0.7	0.0	1.2	0.9	4.3	0.1	8.3	2.9
LC005	I	41.9	224.8	28.2b	19.3	~~106.4~~	14.4	88.5	17.8	140.7	74.3
	II	42.1	230.6	29.2b	22.3	~~104.6~~	14.8	92.6	18.4	156.2	75.7
	s	0.2	4.2	0.7	2.1	~~1.2~~	0.3	2.9	0.4	11.0	1.0
LC006	I	22.0	187.1		8.8	79.0	8.7	70.9	17.0	114.6	56.3
	II	24.5	179.5		9.0	82.0	12.0	69.9	20.1	130.5	57.9
	s	1.8	5.3		0.2	2.1	2.3	0.7	2.2	11.2	1.2
LC007	I	37.1			9.4	38.3	16.5	86.1	46.8	126.3	75.7
	II	33.4			12.9	35.3	16.3	86.3	52.0	106.3	91.0
	s	2.6			2.5	2.1	0.1	0.1	3.7	14.2	10.8
LC009	I	32.2	207.7		15.9	56.1	10.4	59.7	34.4	125.3	
	II	32.5	200.0		12.8	55.0		62.6	35.7	116.7	
	s	0.2	5.4		2.2	0.7		2.0	0.9	6.1	
LC011	I	38.8	164.3	8.9	6.5	47.1	9.8	112.7	8.7	148.8	58.1
	II	38.6	171.8	8.6	7.8	49.4	10.5	110.2		135.7	59.4
	s	0.1	5.3	0.2	1.0	1.6	0.5	1.7		9.2	1.0
LC012	I	~~21.9~~	122.9	9.0	12.3	40.5	~~69.2~~	54.4	32.1	68.9	52.7
	II	~~8.0~~	112.6		8.6	35.8	~~49.7~~	51.8	20.2	72.0	24.9
	s	~~9.8~~	7.3		2.6	3.3	~~13.8~~	1.9	8.4	2.2	19.6
LC013	I	35.4	195.7	3.5	9.6	68.2			32.2	124.4	~~35.5~~
	II		199.7			59.6			23.6	114.8	
	s		2.8			6.1			6.1	6.8	
LC014	I	33.1	231.4		7.8	41.9	15.8	107.3	30.8	92.0	53.0
	II	32.8	233.3		10.3	46.6	16.9	103.1	28.9	88.5	52.3
	s	0.2	1.4		1.8	3.4	0.7	3.0	1.4	2.5	0.5
LC015	I	34.8	154.1		18.3	52.1	20.6	127.2	51.2	169.2	98.4
	II	32.7	148.2		19.7	51.3	14.6	129.5	49.7	166.8	106.5
	s	1.5	4.2		1.0	0.6	4.3	1.6	1.1	1.7	5.7
LC016	I	34.8	154.1		18.3	52.1	20.6	127.2	51.2	169.2	98.4
	II	32.7	148.2		19.7	51.3	14.6	129.5	49.7	166.8	106.5
	s	1.5	4.2		1.0	0.6	4.3	1.6	1.1	1.7	5.7
LC017	I	13.8	102.4		10.9	53.2	20.8	81.2	29.6	94.9	55.3
	II	15.1	96.4			47.7	12.5	71.9	19.3	64.2	57.2
	s	0.9	4.2			3.8	5.8	6.6	7.3	21.7	1.3
LC018	I	24.9	102.2	5.9	8.7	35.5	16.4	~~95.9~~c	37.8	85.3	54.7
	II	19.8	97.9		6.9	31.3	17.5	70.8c	34.9	91.6	47.5
	s	3.6	3.1		1.3	2.9	0.7	17.7	2.0	4.4	5.1

Sample: PM_01											
[µg/kg] Labcode		Hn	HnN	Im/La	Lc	LcN	Re	ReN	Sc	ScN	SpN
LC019	I	37.9	224.8	6.8		57.4		66.7	34.3	70.0	76.5
	II	34.4	207.8	10.0		54.3		63.1	22.1	73.7	58.4
	s	2.5	12.0	2.3		2.1		2.5	8.6	2.6	12.9
LC020	I	30.1	128.8	5.6	17.3	47.9	18.4	88.0	60.7	111.6	73.4
	II	22.5	108.0		9.0	41.2	10.6	76.2	47.1	96.2	61.7
	s	5.4	14.7		5.9	4.7	5.5	8.3	9.7	10.9	8.3
LC023	I	8.4	61.2	8.7		31.1	19.1	74.1	32.1	107.0	75.7
	II	9.0	60.7	8.8		32.5	18.0	78.9	31.5	108.6	73.8
	s	0.4	0.3	0.0		1.0	0.7	3.4	0.4	1.2	1.3
LC025	I	32.7	201.7	7.8	15.0	50.7	27.2	57.6	43.3	135.8	38.4
	II	34.5	205.7	10.0	13.0	41.8	19.3	64.3	48.4	121.4	37.1
	s	1.2	2.8	1.6	1.4	6.3	5.6	4.8	3.6	10.2	0.9
LC118	I	21.9	104.1	6.3		33.1	22.3	~~97.1~~	41.2	86.5	45.3
	II	19.2	84.2			24.0	14.9	~~61.9~~	32.6	67.5	33.9
	s	2.0	14.1			6.4	5.2	24.9	6.1	13.4	8.0

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Sample: PM_02										
[µg/kg] Labcode		Em	Im/La	Re	ReN	Sc	ScN	Sk	Sp	SpN
LC001	I				56.5		175.8	21.3		89.1
	II				56.7		175.5	21.4		79.5
	s				0.2		0.2	0.1		6.8
LC002	I	56.0	46.0	13.5	73.3	113.2	447.0	22.5	46.1	172.6
	II	55.8	45.2	13.2	72.9	111.5	436.5	23.3	48.3	177.6
	s	0.1	0.5	0.2	0.3	1.2	7.4	0.6	1.5	3.5
LC003	I	14.6	41.9	49.8b	107.7	40.5	381.9	~~50.7~~	25.8	143.0
	II	18.5	38.2	57.2b	105.3	38.2	365.2	~~53.2~~	27.2	134.1
	s	2.8	2.6	5.3	1.7	1.6	11.8	~~1.8~~	1.0	6.3
LC004	I	32.1	58.6	9.4	85.3	64.9	303.6	9.7	26.8	144.2
	II	33.1	55.9	10.8	75.7	63.6	277.6	7.7	26.8	138.6
	s	0.7	1.9	1.0	6.8	0.9	18.3	1.4	0.0	4.0
LC005	I	59.1	67.2	17.1	104.2	89.3	460.5	9.6	30.8	203.2
	II	49.3	67.8	19.1	105.8	85.7	482.2	10.8	34.2	215.7
	s	6.9	0.5	1.4	1.1	2.5	15.3	0.8	2.4	8.8
LC006	I		39.1	18.8	119.1	36.5	327.6		27.4	162.8
	II		43.7	17.0	117.9	41.1	314.9		26.2	180.8
	s		3.2	1.2	0.8	3.2	9.0		0.9	12.7
LC007	I	32.7	61.3	20.8	98.9c	86.3	487.8	15.8	41.7	159.7
	II	37.2	59.1	22.8	133.1c	116.4	498.8	13.8	40.7	181.8
	s	3.2	1.6	1.4	24.2	21.2	7.7	1.5	0.7	15.6
LC009	I	44.7	49.4		80.5	69.2	335.6			150.4
	II	46.7	48.5		79.0	68.5	331.8			149.2

Sample: PM_02										
[µg/kg] Labcode		Em	Im/La	Re	ReN	Sc	ScN	Sk	Sp	SpN
	s	1.4	0.7		1.1	0.5	2.7			0.9
LC011	I	27.0	76.9	23.5	126.2	84.9	551.1	18.7	39.9	130.3
	II	27.2	75.6	16.4	123.4	78.1	500.9	19.9	42.1	131.7
	s	0.1	0.9	5.0	2.0	4.8	35.5	0.8	1.6	1.0
LC012	I	~~62.2~~	25.2	~~295.2~~	66.7	88.0	277.5	~~18.1~~	10.7	123.7
	II	~~35.6~~		~~215.8~~	51.4	69.0	244.1	~~10.0~~		90.7
	s	~~18.8~~		~~56.2~~	10.8	13.4	23.6	~~5.7~~		23.3
LC013	I	18.4	31.0			38.4	152.5	11.6		~~45.9~~
	II	16.3	20.7			30.3	157.2	11.2		
	s	1.5	7.3			5.7	3.3	0.3		
LC014	I	8.9	60.6	14.8	67.2	80.9	292.3	22.4	68.0	172.2
	II	9.6	62.6	15.3	71.0	80.9	296.9	24.9	73.4	167.1
	s	0.5	1.4	0.4	2.7	0.0	3.3	1.8	3.8	3.6
LC015	I		49.2	11.9	86.9	62.8	280.5	5.9	33.7	153.6
	II		64.6	13.0	93.7	69.3	323.0	10.2	41.9	181.7
	s		10.9	0.8	4.8	4.6	30.0	3.0	5.8	19.9
LC016	I		90.4	11.4	118.3	97.1	455.8	14.3		183.7
	II		79.4	14.6	124.3	89.7	424.9	15.6		202.5
	s		7.8	2.2	4.3	5.2	21.8	1.0		13.3
LC017	I	13.3	61.4	16.6	94.5	61.3	379.6	25.5	34.0	196.2
	II	7.7	42.7	14.3	96.2	46.8	406.3	26.2	33.9	189.7
	s	4.0	13.2	1.6	1.2	10.3	18.9	0.5	0.1	4.6
LC018	I	7.3	67.9	21.5	128.3	88.7	383.7	34.3	36.7	188.3
	II	9.7	65.4	18.6	130.2	98.9	380.7	27.9	29.1	197.2
	s	1.8	1.8	2.0	1.3	7.2	2.1	4.5	5.4	6.3
LC019	I	32.4	28.8		96.8	116.4	351.5	17.3	44.8	208.2
	II	30.4	37.7		102.8	103.6	343.3	16.3	41.7	177.2
	s	1.4	6.2		4.3	9.1	5.8	0.7	2.2	21.9
LC020	I	8.2	70.1	19.2	126.2	133.8	325.3	26.7	37.2	179.0
	II		52.9	11.9	107.2	117.1	293.6	22.9	28.6	159.3
	s		12.2	5.2	13.4	11.8	22.4	2.7	6.1	14.0
LC023	I	5.6	42.2	10.1	79.6	57.7	249.2	9.1	36.5	148.0
	II	5.2	41.8	8.4	78.3	55.5	243.0	9.5	37.6	152.4
	s	0.3	0.3	1.2	0.9	1.5	4.4	0.3	0.7	3.1
LC025	I		55.5	11.3	105.2	77.4	330.7	26.4	22.6	195.6
	II		55.0	13.8	106.2	74.7	310.1	23.3	27.6	196.2
	s		0.3	1.8	0.7	1.9	14.6	2.2	3.6	0.4
LC118	I		102.8	21.4	~~143.6~~	140.5	472.1	14.2	41.9	193.7
	II		112.2	14.3	~~118.5~~	148.6	418.5	7.4	38.3	188.9
	s		6.7	5.0	~~17.7~~	5.7	37.9	4.8	2.5	3.4

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Sample: PM_03						
[µg/kg] Labcode		Im/La	Re	ReN	Sc	ScN
LC001	I	7.3		11.1		41.6
	II	9.3		10.0		45.1
	s	1.4		0.7		2.5
LC002	I	60.1	42.0	36.6	82.3	88.1
	II	58.9	39.9	37.2	77.6	80.9
	s	0.8	1.5	0.4	3.3	5.1
LC003	I	34.4	41.7	41.3	27.9	60.1
	II	30.6	35.1	52.2	26.4	70.7
	s	2.7	4.7	7.7	1.1	7.5
LC004	I	245.8b	28.5	52.0	40.9	70.9
	II	241.2b	30.3	46.1	42.9	65.3
	s	3.3	1.3	4.2	1.5	4.0
LC005	I	80.5	24.6	52.5	63.9	151.1
	II	80.6	26.6	48.1	58.8	162.1
	s	0.1	1.4	3.1	3.6	7.8
LC006	I	24.7	13.4	17.0	19.8	64.2
	II	27.5	10.6	16.3	23.2	67.6
	s	2.0	2.0	0.5	2.4	2.4
LC007	I		6.7	1.1	4.7	8.4
	II		30.2	42.7	47.5	100.2
	s		20.7	41.1	54.2	88.3
LC009	I	46.9	15.0	19.6	50.1	
	II	47.1	8.9	23.5	47.4	
	s	0.1	4.4	2.7	2.0	
LC011	I	35.5	28.7	48.0	60.1	121.8
	II	33.1	30.0	47.5	54.9	114.2
	s	1.7	0.9	0.4	3.7	5.4
LC012	I	33.7	~~95.9~~	31.4	53.4	85.2
	II	16.4	~~57.2~~	17.6	37.2	74.7
	s	12.3	~~27.4~~	9.7	11.5	7.4
LC013	I	13.0	11.1		34.7	57.5
	II	7.7	9.9		24.1	57.6
	s	3.8	0.8		7.5	0.1
LC014	I			10.5	10.9	32.6
	II			16.4	10.7	32.4
	s			4.1	0.1	0.2
LC015	I	10.0	18.2	36.0	54.0	71.4
	II		21.8	36.4	67.2	84.6
	s		2.6	0.3	9.3	9.3
LC016	I	45.4	21.1	39.8	73.7	66.0
	II	42.0	19.1	41.8	73.1	71.2
	s	2.4	1.4	1.5	0.4	3.6
LC017	I		21.1	42.7	42.9	59.7
	II		14.9	31.8	31.4	49.0
	s		4.4	7.7	8.1	7.5
LC018	I	25.7	12.0	27.1	35.2	46.3
	II	8.1	16.9	28.0	33.6	51.0
	s	12.4	3.5	0.7	1.1	3.3

Sample: PM_03						
[µg/kg] Labcode		Im/La	Re	ReN	Sc	ScN
LC019	I	42.8	25.0	47.1	59.0	80.3
	II	48.6	27.8	26.5	58.1	78.8
	s	4.1	2.0	14.6	0.7	1.1
LC020	I	30.6	22.4	28.5	93.3	78.9
	II	14.7	16.0	19.1	80.2	69.4
	s	11.2	4.6	6.6	9.3	6.8
LC023	I	38.0	28.7	48.1	53.3	76.1
	II	42.5	26.7	51.2	51.2	75.8
	s	3.2	1.4	2.2	1.5	0.2
LC025	I	43.4	23.6	19.3	46.7	116.6
	II	45.8	21.8	25.4	49.2	121.9
	s	1.7	1.3	4.3	1.7	3.8
LC118	I	30.7	14.8	~~31.6~~	47.0	56.8
	II	19.0	7.3	~~13.7~~	38.2	52.6
	s	8.3	5.4	~~12.7~~	6.2	3.0

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

Sample: PM_04					
[µg/kg] Labcode		Re	ReN	Sc	ScN
LC001	I		32.4		160.8
	II		31.9		159.4
	s		0.3		1.0
LC002	I	61.2	76.9	116.3	304.7
	II	60.2	65.1	120.6	313.4
	s	0.7	8.3	3.0	6.2
LC003	I	47.9	158.4	72.8	251.0
	II	50.6	160.1	58.6	265.9
	s	1.9	1.2	10.0	10.5
LC004	I	51.2	182.5	57.5	429.5
	II	50.0	164.8	52.6	390.7
	s	0.9	12.5	3.5	27.4
LC005	I	45.2	153.2	128.0	543.8
	II	47.3	159.4	134.3	534.9
	s	1.5	4.4	4.5	6.3
LC006	I	18.4	88.8	27.6	283.7
	II	21.3	90.4	28.5	279.2
	s	2.1	1.1	0.6	3.2
LC007	I	41.8	140.1	83.0	400.2
	II	37.5	126.7	112.2	378.2
	s	3.0	9.5	20.6	15.6
LC009	I		62.4	74.7	306.7
	II		67.0	67.5	305.3
	s		3.3	5.1	1.0

Sample: PM_04					
[µg/kg] Labcode		Re	ReN	Sc	ScN
LC011	I	57.7	155.3	124.2	685.9
	II	56.6	151.6	128.0	624.6
	s	0.8	2.6	2.7	43.4
LC012	I	~~233.5~~	74.7	80.4	206.4
	II	~~143.6~~	68.6	60.2	184.1
	s	~~63.6~~	4.3	14.3	15.7
LC013	I	15.1		55.2	179.8
	II	13.3		48.2	179.7
	s	1.3		4.9	0.0
LC014	I	5.3	32.6	17.3	73.5
	II	5.6	33.4	20.1	77.3
	s	0.2	0.6	1.9	2.7
LC015	I	32.0	92.3	91.3	252.4
	II	33.3	97.6	105.6	327.1
	s	0.9	3.7	10.1	52.9
LC016	I	22.0	118.9	68.3	306.9
	II	26.3	110.1	75.3	307.7
	s	3.1	6.2	5.0	0.6
LC017	I	32.1	88.7	55.6	144.1
	II	18.2	80.7	50.3	163.7
	s	9.9	5.7	3.7	13.8
LC018	I	20.2	57.3	51.9	168.4
	II	16.7	53.9	48.3	152.4
	s	2.5	2.4	2.6	11.3
LC019	I	34.2	92.1	81.7	241.6
	II	24.5	117.7	88.7	279.1
	s	6.9	18.1	4.9	26.5
LC020	I	25.0	58.7	102.1	223.6
	II	19.0	51.7	87.9	192.0
	s	4.2	5.0	10.1	22.4
LC023	I	27.0	137.3	28.5	320.2
	II	24.1	143.3	25.1	329.3
	s	2.0	4.2	2.4	6.5
LC025	I	55.9	149.4	99.6	339.8
	II	51.1	166.0	83.2	323.5
	s	3.4	11.7	11.6	11.5
LC118	I	17.5	~~99.0~~	93.7	243.2
	II	11.4	~~69.9~~	76.9	208.6
	s	4.3	~~20.6~~	11.8	24.5

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

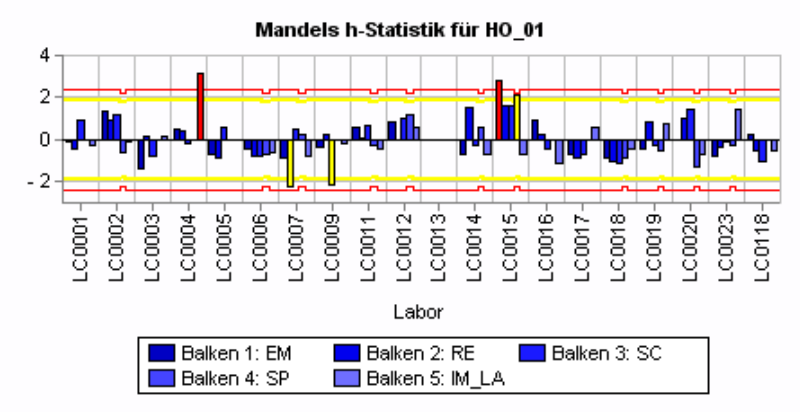
Sample: PM_05											
[µg/kg]		Hn	HnN	Im/La	Lc	LcN	Re	ReN	Sc	ScN	SpN
Labcode											
LC001	I		~~65.1~~			21.4		39.5		57.8	31.32
	II		~~61.7~~			18.9		36.9		58.6	31.61
	s		~~2.4~~			1.8		1.9		0.6	0.2
LC002	I	33.8	140.7	11.7	~~26.9~~	62.1	28.6	104.4	50.2	123.8	95.87
	II	33.5	139.8	11.3	~~26.7~~	60.1	30.6	106.0	48.6	119.9	94.78
	s	0.2	0.6	0.3	~~0.1~~	1.4	1.4	1.1	1.2	2.7	0.8
LC003	I	41.0	149.1	8.2	9.2	29.6	30.7	85.4	19.7	92.6	73.8
	II	40.5	125.9	8.4	7.7	21.1	40.3	100.6	16.1	97.0	80.4
	s	0.3	16.4	0.1	1.1	6.0	6.8	10.7	2.6	3.2	4.6
LC004	I	39.3	178.7	35.1	7.4	73.1	17.5	71.6	11.4	77.7	71.2
	II	39.1	170.3	36.1	8.4	65.4	19.0	65.4	9.9	76.4	67.6
	s	0.1	6.0	0.7	0.8	5.4	1.0	4.4	1.1	0.9	2.5
LC005	I	26.3	170.9	36.1	9.1	~~80.2~~	10.1	88.5	21.8	164.1	88.4
	II	24.7	180.1	35.7	14.2	~~84.5~~	11.2	94.9	22.1	175.2	96.9
	s	1.1	6.5	0.3	3.6	~~3.0~~	0.7	4.5	0.2	7.9	6.0
LC006	I	35.4	245.9		9.3	65.6	23.5	77.0	21.9	91.9	51.5
	II	34.5	231.9		10.1	76.0	21.3	64.6	23.9	101.9	58.7
	s	0.6	9.9		0.6	7.4	1.6	8.8	1.4	7.1	5.1
LC007	I	68.0			13.8	60.0	30.1	75.9	36.0	59.2	76.2
	II	69.4	251.7		17.3	59.1		82.2	42.4	75.8	84.2
	s	1.0			2.5	0.7		4.4	4.5	11.7	5.7
LC009	I	32.5	190.1		15.1	56.9	14.4	51.0	42.2	104.6	32.5
	II	32.5	185.5		12.6	49.9	7.9	49.3	40.1	100.2	32.5
	s	0.0	3.3		1.7	5.0	4.6	1.2	1.5	3.1	0.0
LC011	I	29.1	111.9	26.5	7.7	39.5	23.5	73.3	37.6	163.7	49.4
	II	28.2	114.0	18.8	9.3	40.1	21.8	72.0	34.1	150.1	50.3
	s	0.6	1.5	5.5	1.1	0.4	1.2	0.9	2.5	9.6	0.7
LC012	I	~~21.8~~	146.3	18.3	15.4	52.2	~~114.0~~	74.9	36.2	110.1	65.4
	II	~~6.9~~	126.7		9.9	41.0	~~66.5~~	52.9	18.2	85.5	39.9
	s	~~10.6~~	13.8		3.9	7.9	~~33.6~~	15.6	12.7	17.4	18.0
LC013	I	16.7	107.9	1.1		50.1			42.7	177.8	~~113.6~~
	II	13.4	104.8			37.4			36.9	178.6	~~66.6~~
	s	2.3	2.2			9.0			4.1	0.5	~~33.2~~
LC014	I	26.9	157.5		10.7	29.9	14.8	82.3	29.4	97.5	54.9
	II	26.4	158.3		13.2	33.3	14.3	86.6	29.6	98.2	57.3
	s	0.3	0.6		1.7	2.5	0.4	3.0	0.2	0.4	1.7
LC015	I	36.7	201.5		9.7	40.6	21.8	70.0	40.9	106.1	80.9
	II	45.3	251.3		13.5	52.3	22.3	80.4	48.2	124.0	95.9
	s	6.1	35.2		2.7	8.3	0.3	7.4	5.2	12.6	10.6
LC016	I	16.5	82.1		11.8	30.6	20.1	108.6	63.4	142.3	76.5
	II	17.3	77.8		11.7	30.4	27.6	102.7	52.0	132.7	75.0
	s	0.5	3.0		0.1	0.2	5.3	4.2	8.1	6.7	1.1
LC017	I	21.2	123.7		11.6	52.5	20.7	71.2	31.3	75.7	55.4
	II	20.1	124.3		5.0	47.5	13.2	66.2	21.5	69.1	52.4
	s	0.7	0.5		4.6	3.6	5.3	3.5	6.9	4.7	2.1
LC018	I	54.6	198.3		14.1	46.3	16.9	72.3	30.6	83.0	56.9
	II	48.7	188.4		11.5	39.3	18.1	70.8	31.1	90.7	49.5
	s	4.2	7.0		1.8	4.9	0.8	1.1	0.3	5.5	5.2

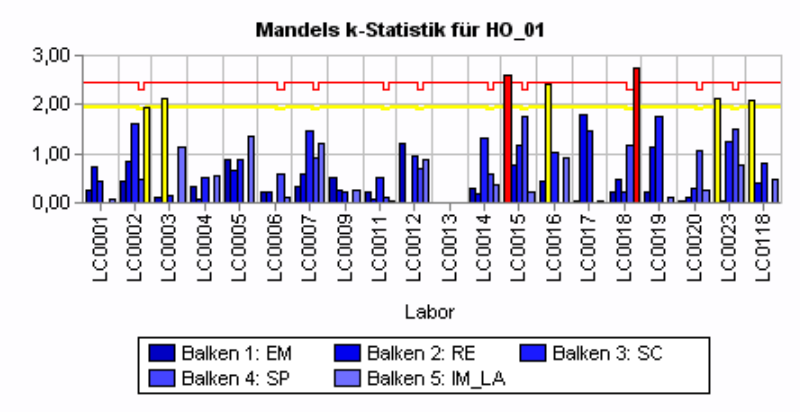
Sample: PM_05											
[µg/kg] Labcode		Hn	HnN	Im/La	Lc	LcN	Re	ReN	Sc	ScN	SpN
LC019	I	32.9	114.6			42.5	18.4	71.7	36.8	85.3	71.8
	II	37.2	134.7	8.9		45.6	20.9	79.7	35.5	97.9	60.7
	s	3.0	14.3			2.2	1.7	5.6	0.9	8.9	7.8
LC020	I	40.0	161.4	11.4	20.7	73.5	21.5	75.9	67.7	96.1	61.6
	II	29.8	138.6	6.6	11.9	65.0	13.8	62.8	57.6	82.6	48.2
	s	7.2	16.1	3.5	6.2	6.0	5.5	9.3	7.1	9.5	9.5
LC023	I	19.4	106.4	10.1		31.6	20.4	82.8	35.9	123.0	79.3
	II	19.9	106.2	9.7		32.6	19.1	88.8	34.9	121.2	78.3
	s	0.3	0.2	0.3		0.7	1.0	4.2	0.7	1.3	0.7
LC025	I	26.5	144.8		12.8	39.7	26.5	100.4	38.9	166.8	52.3
	II	31.2	142.5		11.8	36.3	33.8	104.0	32.3	146.1	52.6
	s	3.4	1.6		0.7	2.4	5.2	2.6	4.7	14.6	0.2
LC118	I	50.4	186.8			46.1	19.8	~~89.3~~	31.1	82.3	74.0
	II	40.2	156.4			33.4	12.6	~~73.9~~	25.2	78.9	49.8
	s	7.2	21.5			9.0	5.1	~~10.9~~	4.2	2.4	17.1

crossed out value: conspicuous and removed value during first Mandel h and k statistic
b: Grubb outlier
c: Cochran outlier
I: first injection value
II: second injection value
s: standard deviation

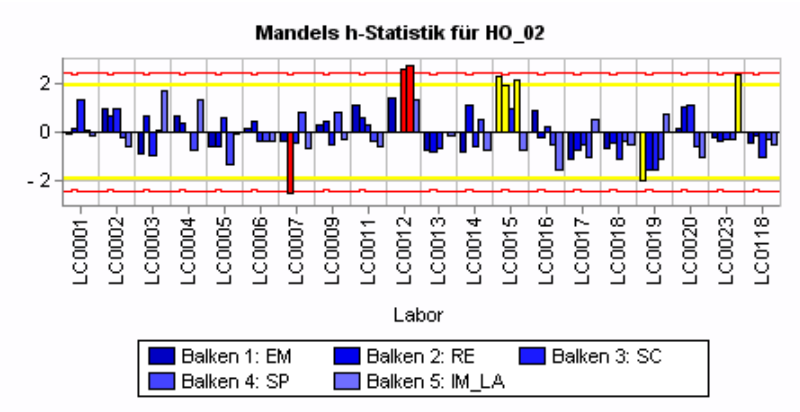
Annex F Mandel h and k statistic diagrams for honey

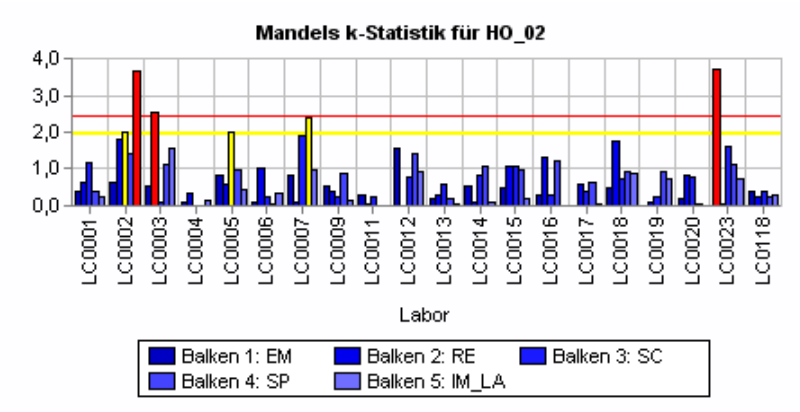
Sample HO_01



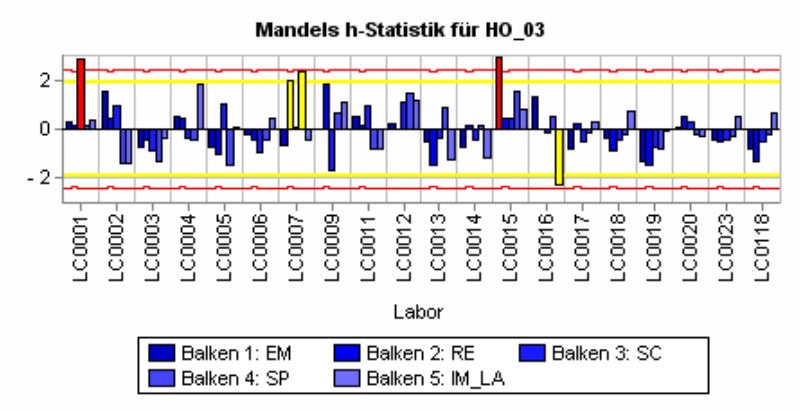


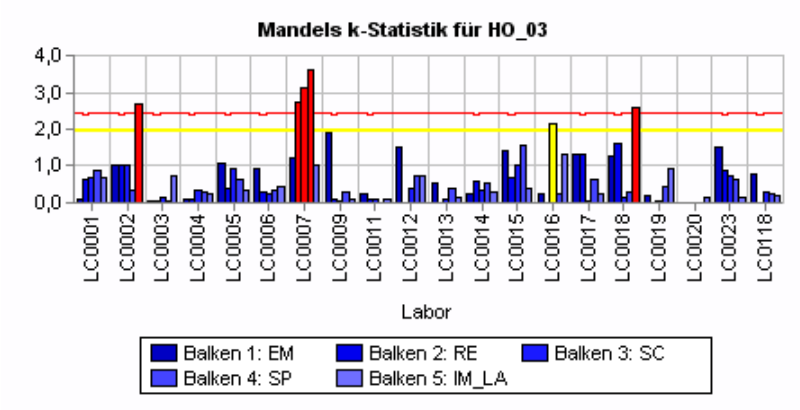
Sample HO_02



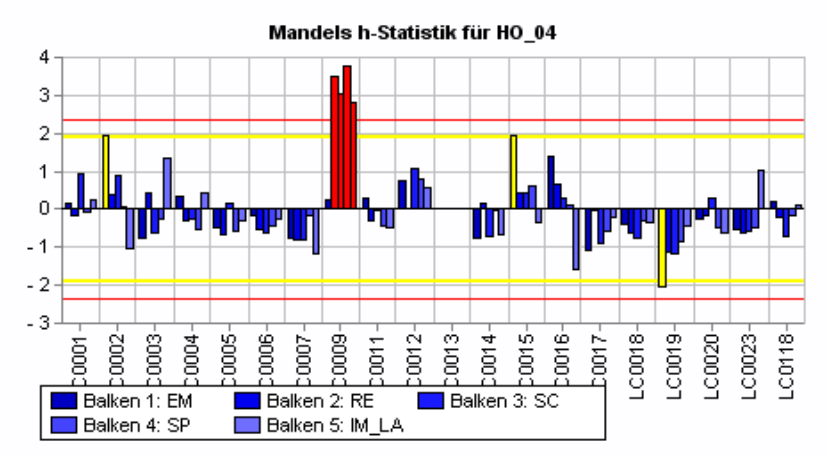


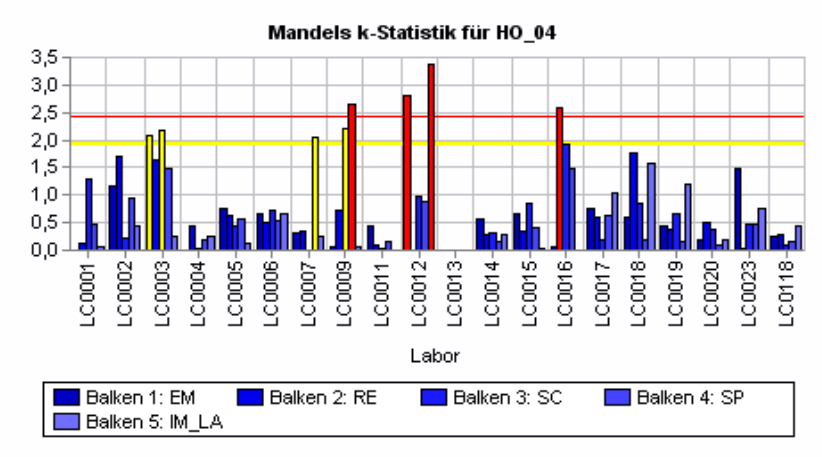
Sample HO_03



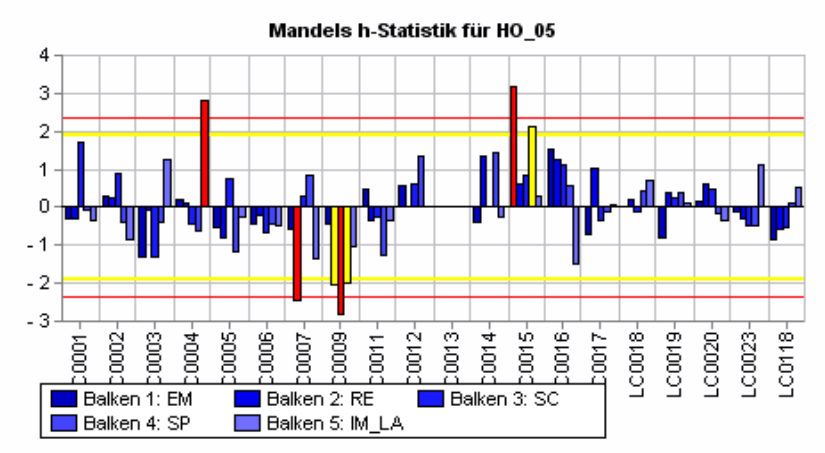


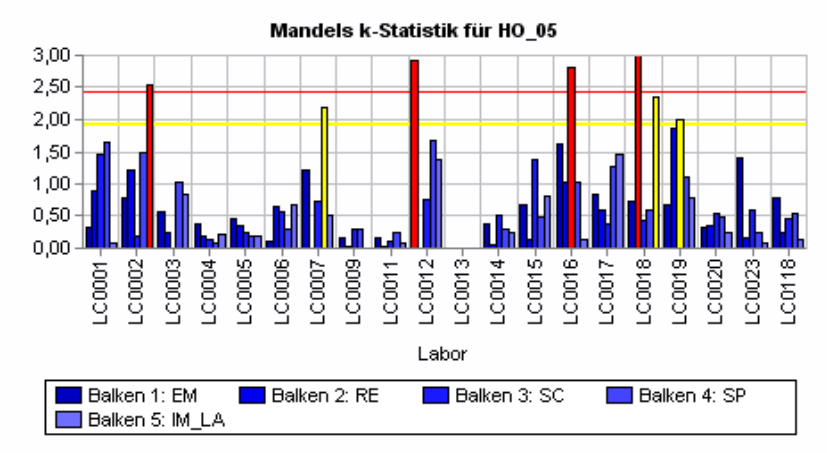
Sample HO_04



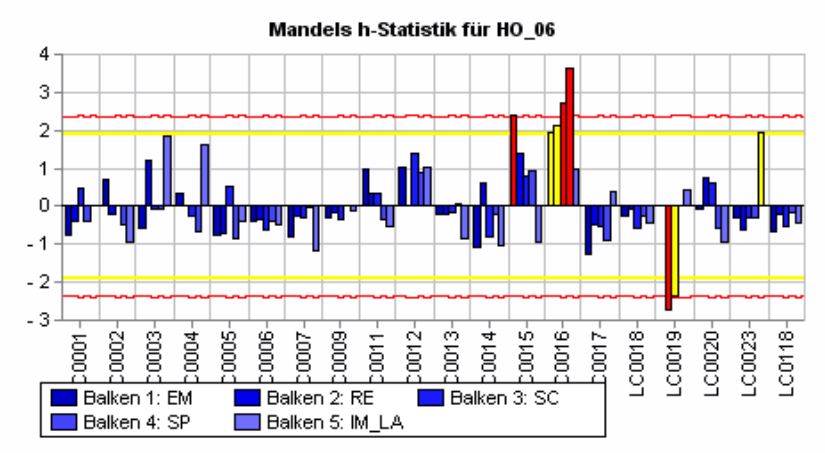


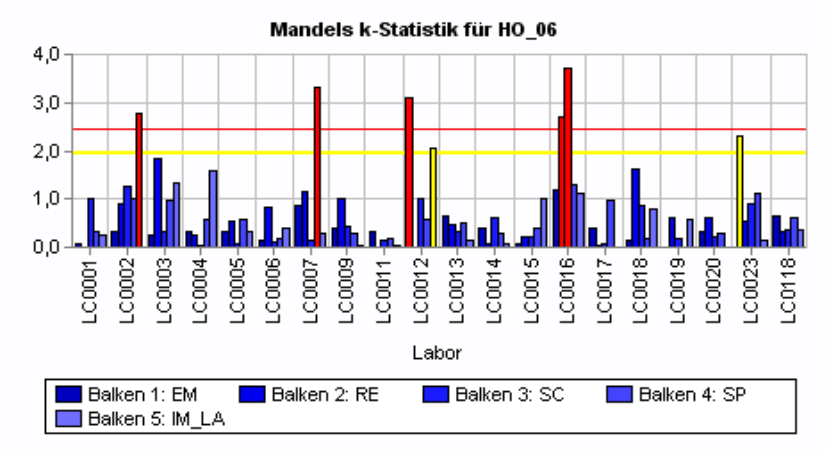
Sample HO_05



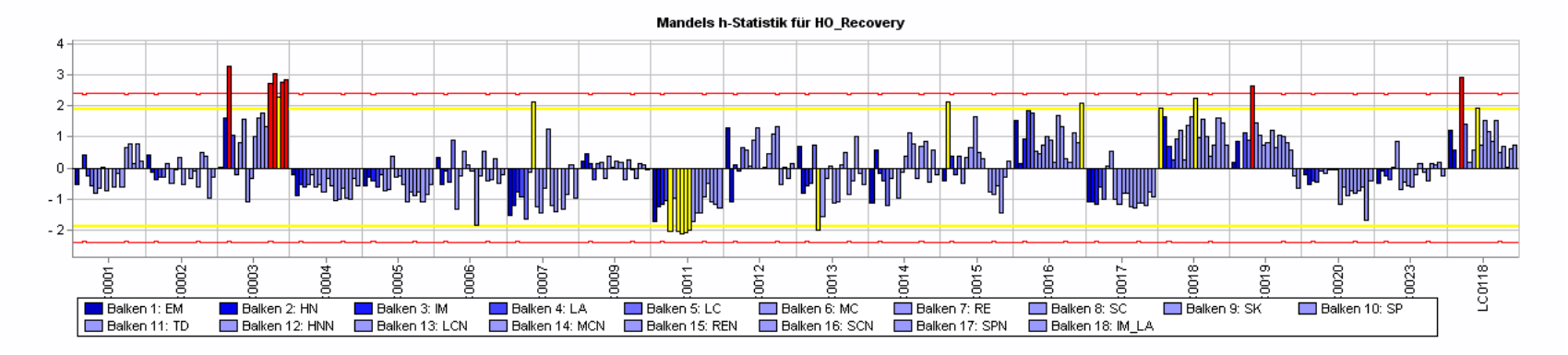


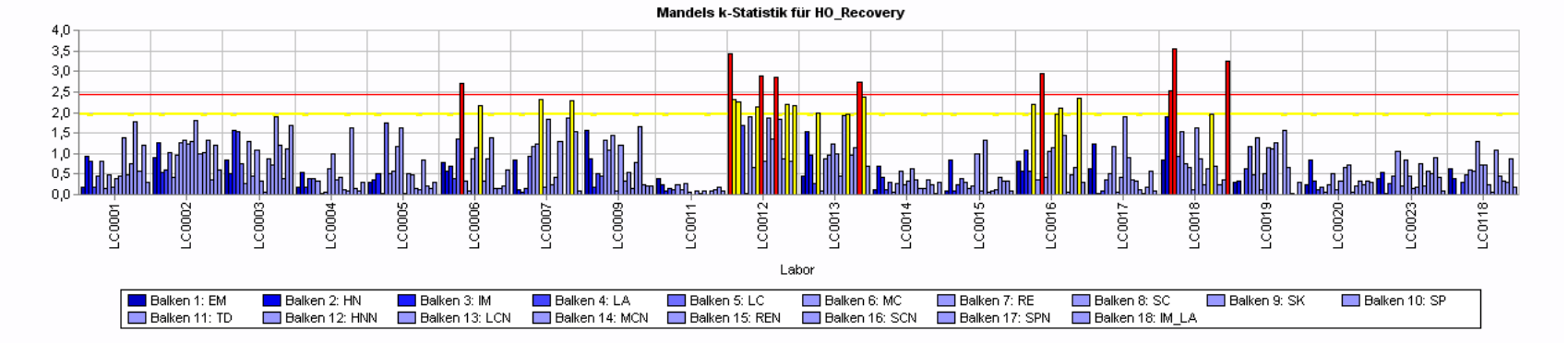
Sample HO_06





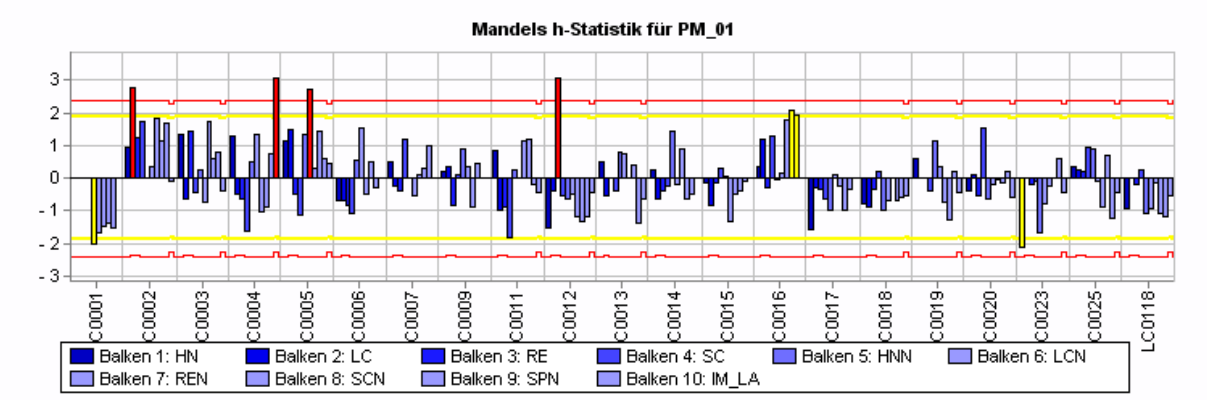
Sample HO_Recovery

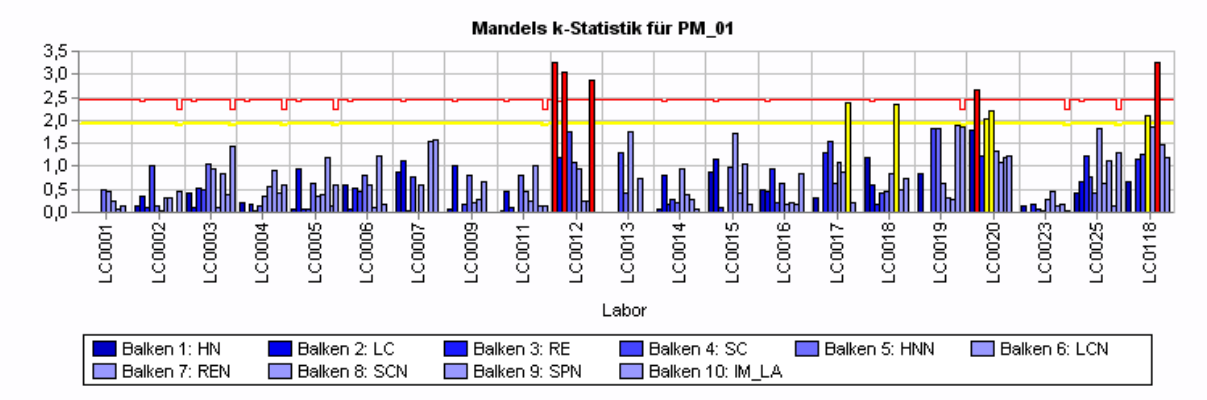




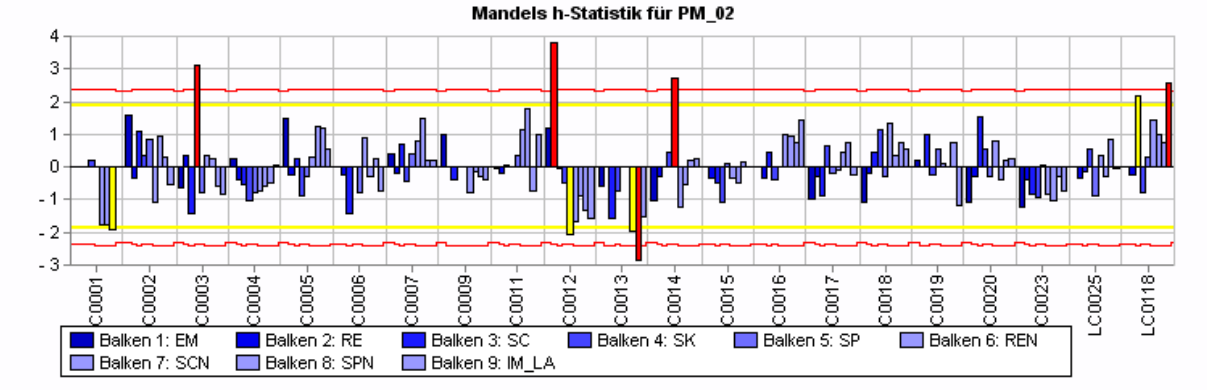
Annex G Mandel h and k statistic diagrams for tea

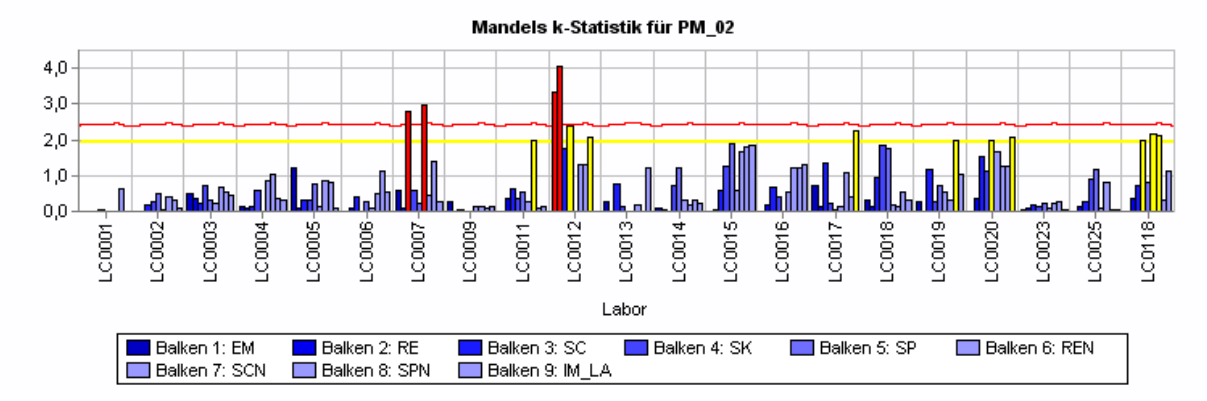
PM_01





PM_02

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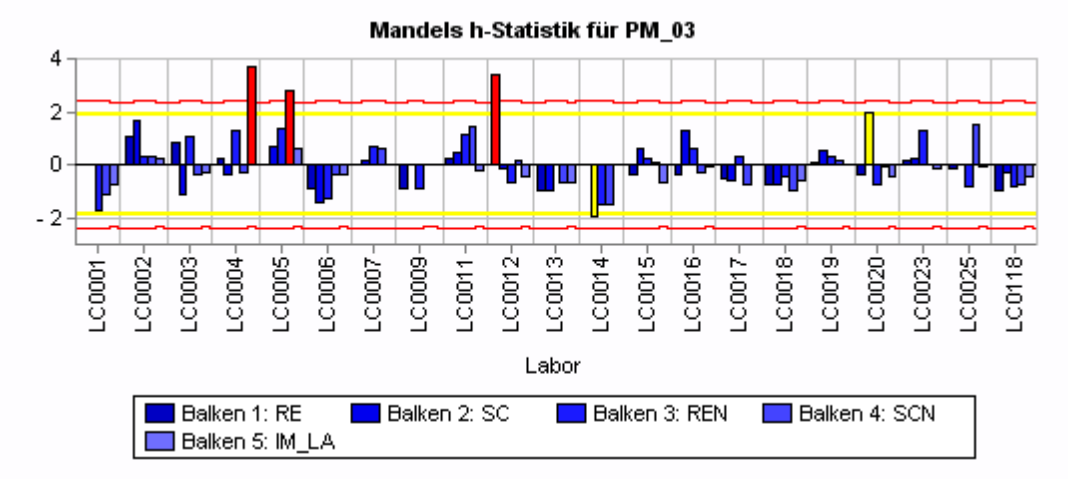
LC0007
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: EM
Balken 2: RE
Balken 3: SC
Balken 4: SK
Balken 5: SP
Balken 6: REN
Balken 7: SCN
Balken 8: SPN
Balken 9: IM_LA

PM_03


Mandels h-Statistik für PM_03
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2
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LC0003
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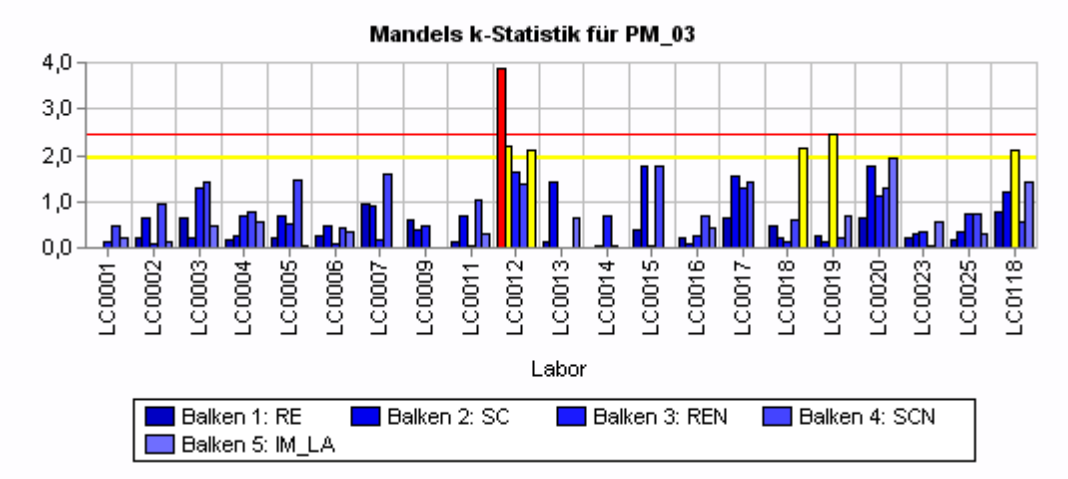
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: RE
Balken 2: SC
Balken 3: REN
Balken 4: SCN
Balken 5: IM_LA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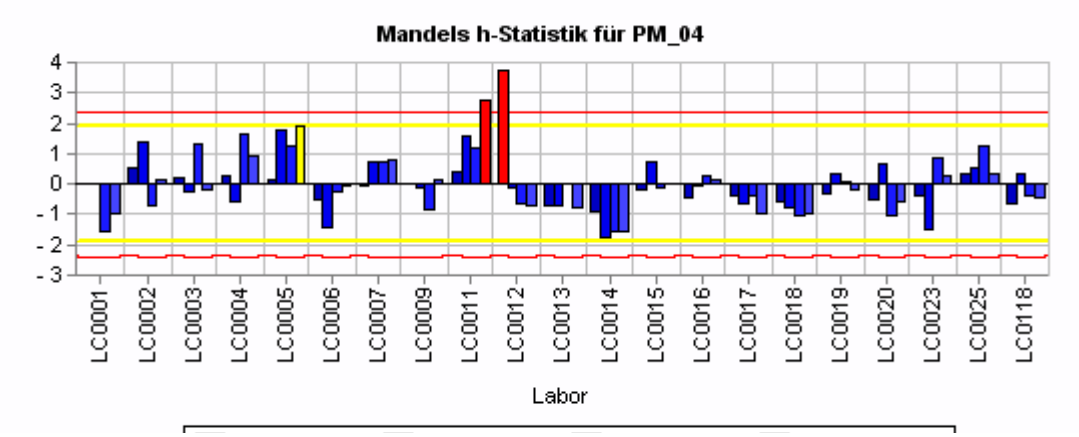
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: RE
Balken 2: SC
Balken 3: REN
Balken 4: SCN
Balken 5: IM_LA

PM_04


Mandels h-Statistik für PM_04
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3
2
1
0
- 1
- 2
- 3
LC0001
LC0002
LC0003
LC0004
LC0005
LC0006
LC0007
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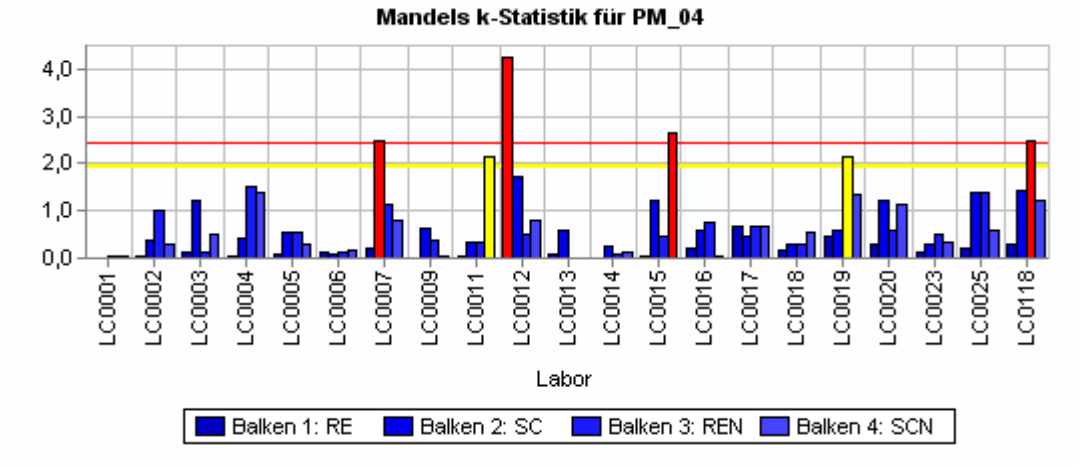
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor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LC0007
LC0009
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LC0012
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LC0014
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LC0017
LC0018
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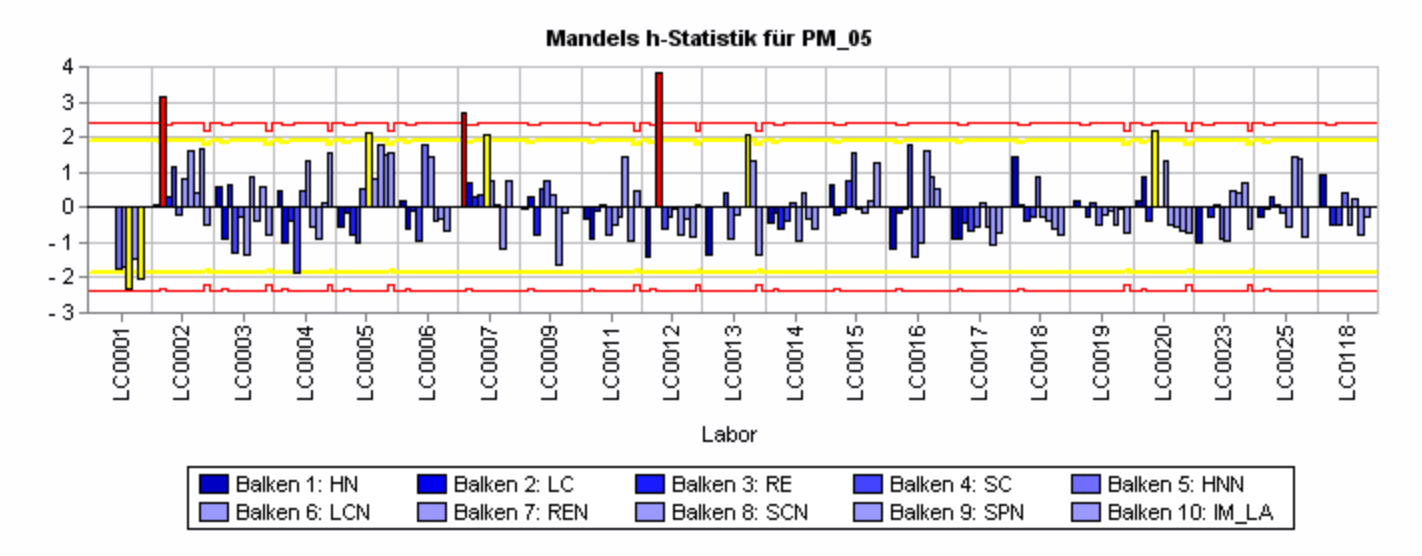
LC0118
Labor
Balken 1: RE
Balken 2: SC
Balken 3: REN
Balken 4: SCN

PM_05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- 1
- 2
- 3
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LC0003
LC0004
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LC0007
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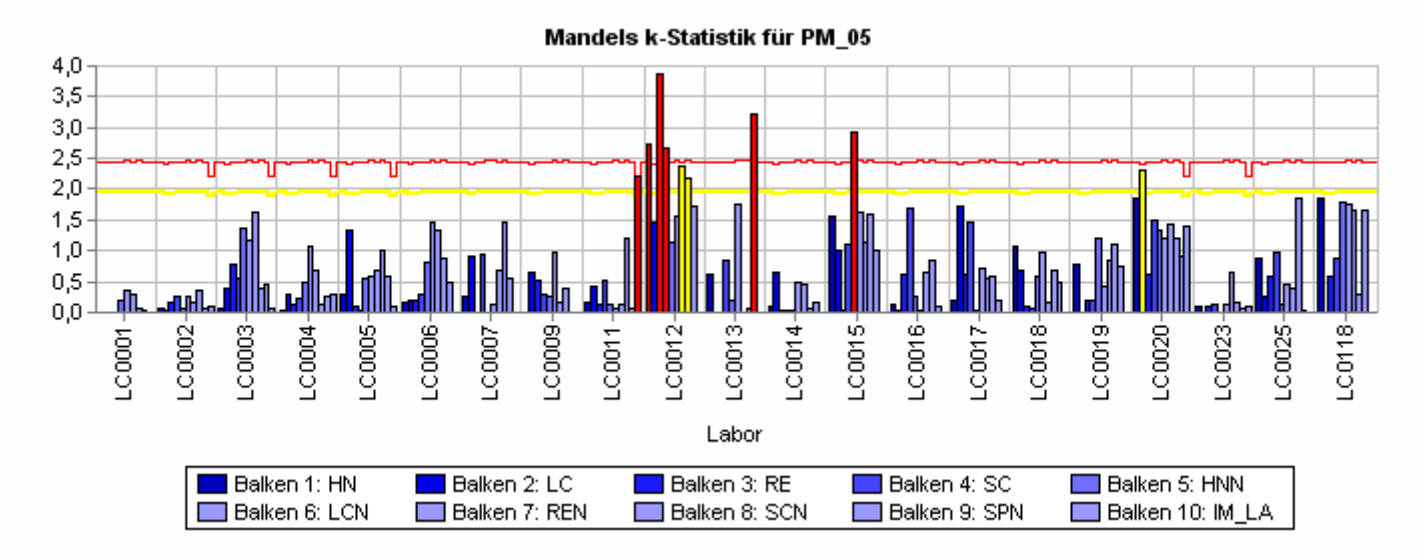
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: HN
Balken 2: LC
Balken 3: RE
Balken 4: SC
Balken 5: HNN
Balken 6: LCN
Balken 7: REN
Balken 8: SCN
Balken 9: SPN
Balken 10: IM_LA


Mandels k-Statistik für PM_05
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2,0
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1,0
0,5
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LC0001
LC0002
LC0003
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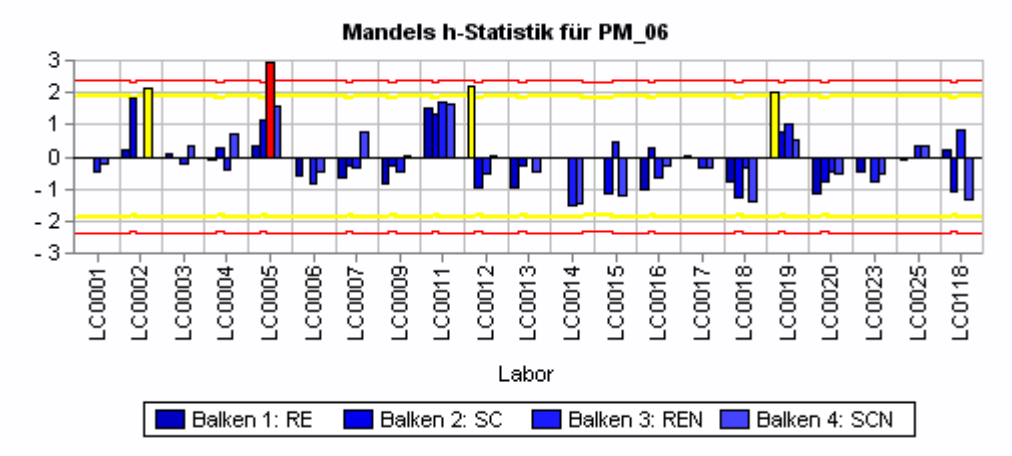
LC0007
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: HN
Balken 2: LC
Balken 3: RE
Balken 4: SC
Balken 5: HNN
Balken 6: LCN
Balken 7: REN
Balken 8: SCN
Balken 9: SPN
Balken 10: IM_LA

PM_06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LC0011
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LC0013
LC0014
LC0015
LC0016
LC0017
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LC0019
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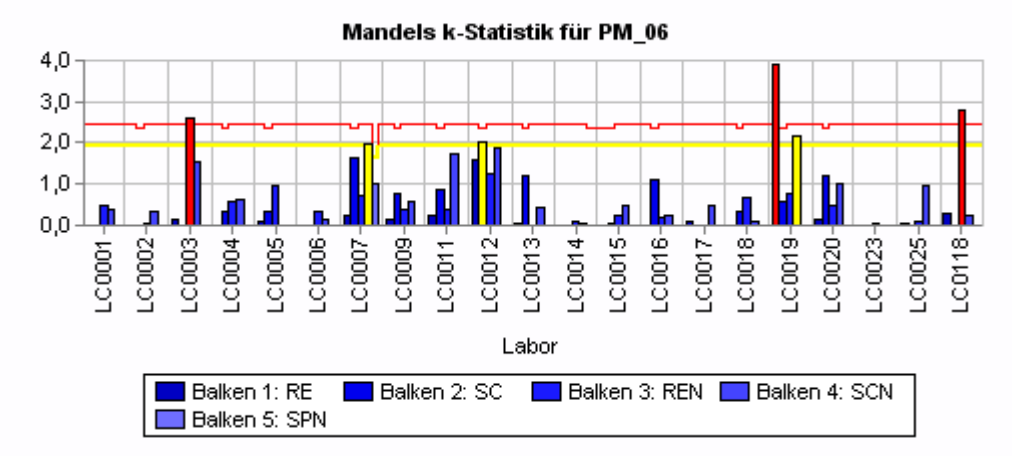
LC0023
LC0025
LC0118
Labor
Balken 1: RE
Balken 2: SC
Balken 3: REN
Balken 4: SCN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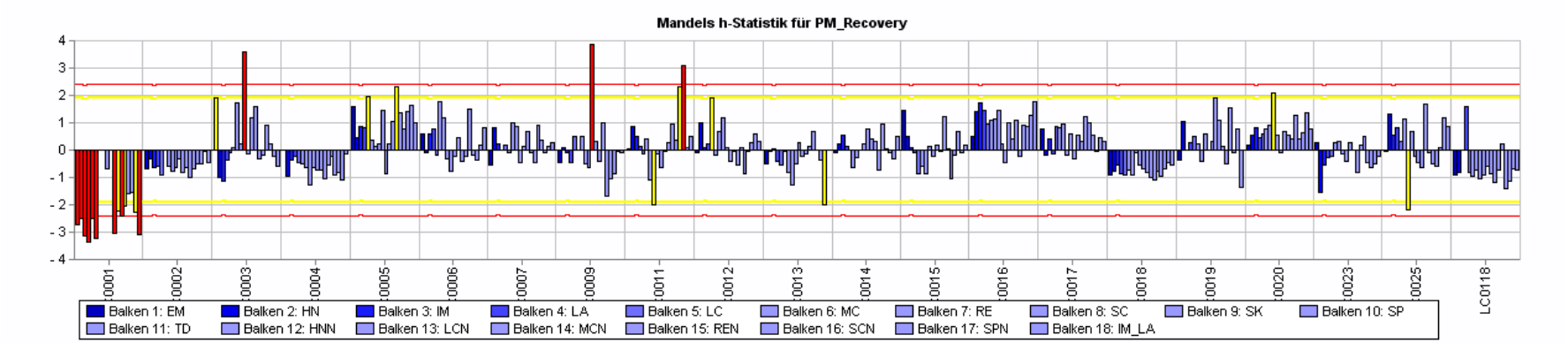
LC0007
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: RE
Balken 2: SC
Balken 3: REN
Balken 4: SCN
Balken 5: SPN

PM_recovery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1
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-1
-2
-3
-4
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LC0002
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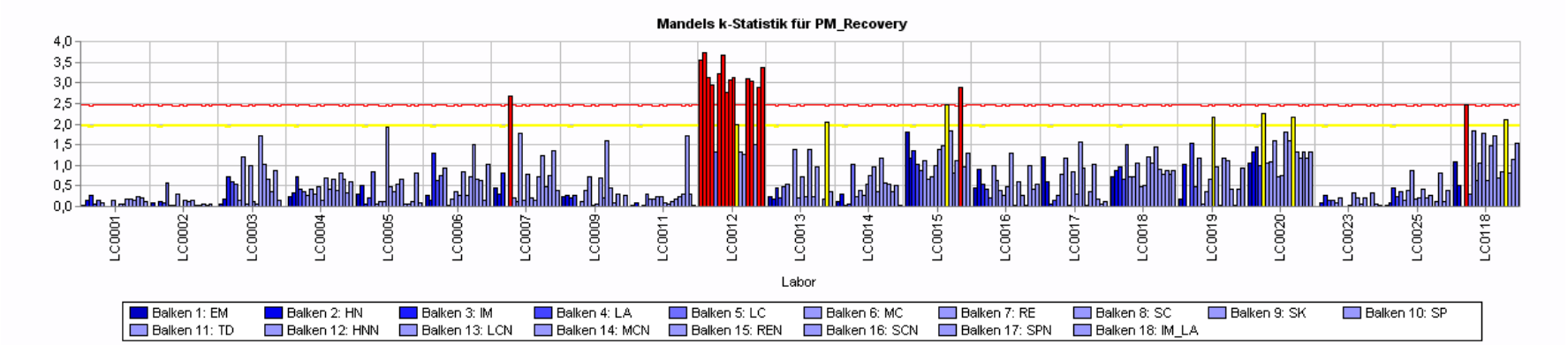
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Balken 1: EM
Balken 2: HN
Balken 3: IM
Balken 4: LA
Balken 5: LC
Balken 6: MC
Balken 7: RE
Balken 8: SC
Balken 9: SK
Balken 10: SP
Balken 11: TD
Balken 12: HNN
Balken 13: LCN
Balken 14: MCN
Balken 15: REN
Balken 16: SCN
Balken 17: SPN
Balken 18: IM_LA


Mandels k-Statistik für PM_Recovery
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1,0
0,5
0,0
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LC0002
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LC0007
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LC0011
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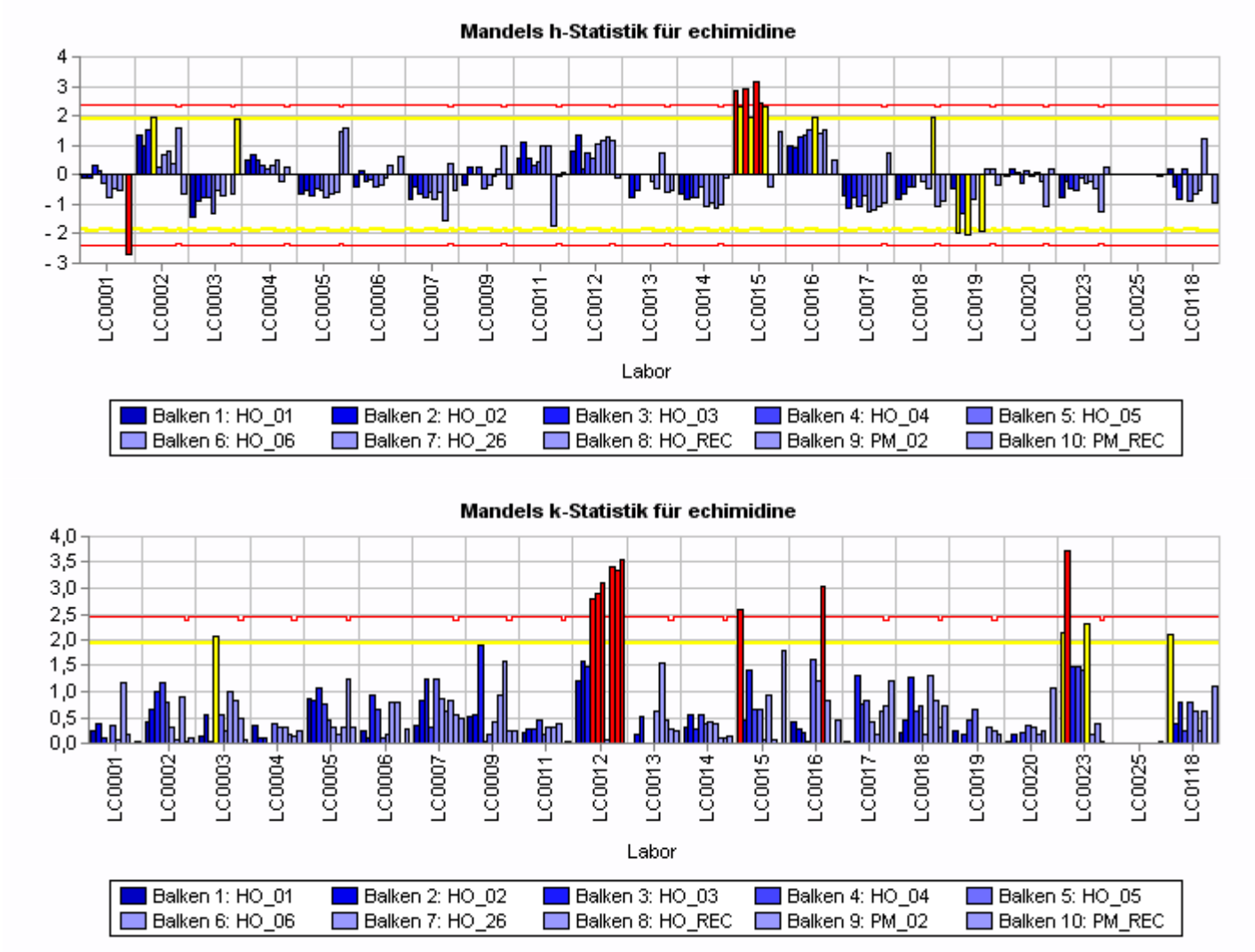
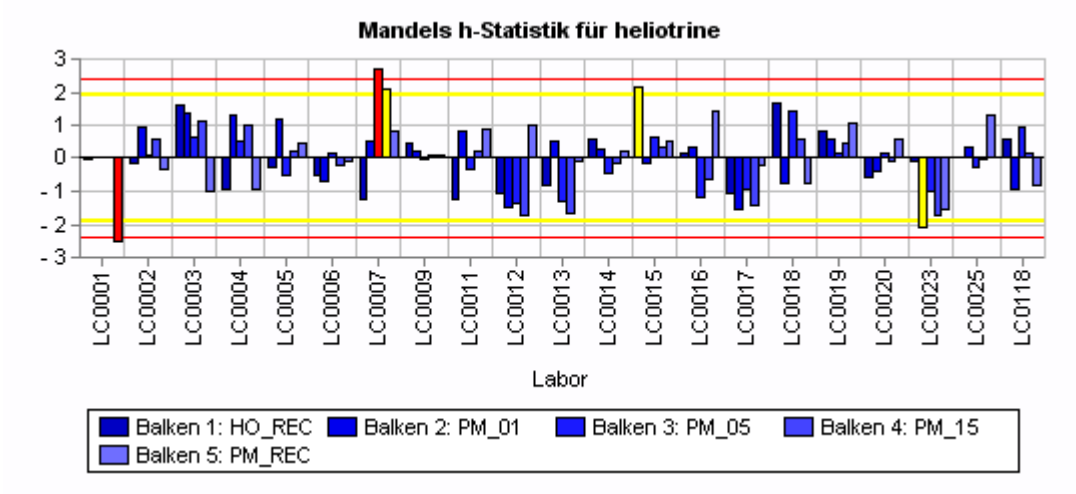
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: EM
Balken 2: HN
Balken 3: IM
Balken 4: LA
Balken 5: LC
Balken 6: MC
Balken 7: RE
Balken 8: SC
Balken 9: SK
Balken 10: SP
Balken 11: TD
Balken 12: HNN
Balken 13: LCN
Balken 14: MCN
Balken 15: REN
Balken 16: SCN
Balken 17: SPN
Balken 18: IM_LA

Annex H Mandel h and k statistic diagrams for individual pyrrolizidine alkaloids

Echimidine



Heliotrine


Mandels h-Statistik für heliotrine
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2
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0
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- 2
- 3
LC0001
LC0002
LC0003
LC0004
LC0005
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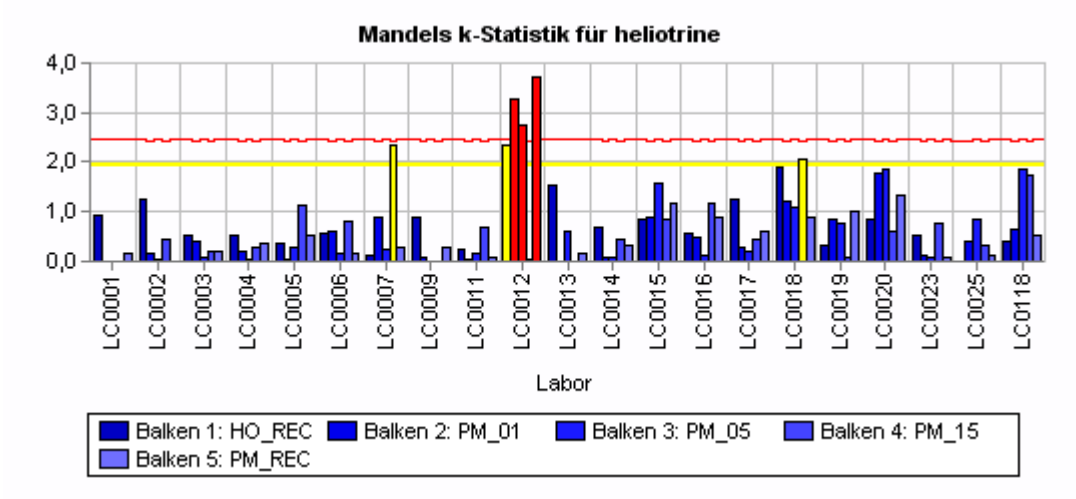
LC0007
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: HO_REC
Balken 2: PM_01
Balken 3: PM_05
Balken 4: PM_15
Balken 5: PM_REC


Mandels k-Statistik für heliotrine
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2,0
1,0
0,0
LC0001
LC0002
LC0003
LC0004
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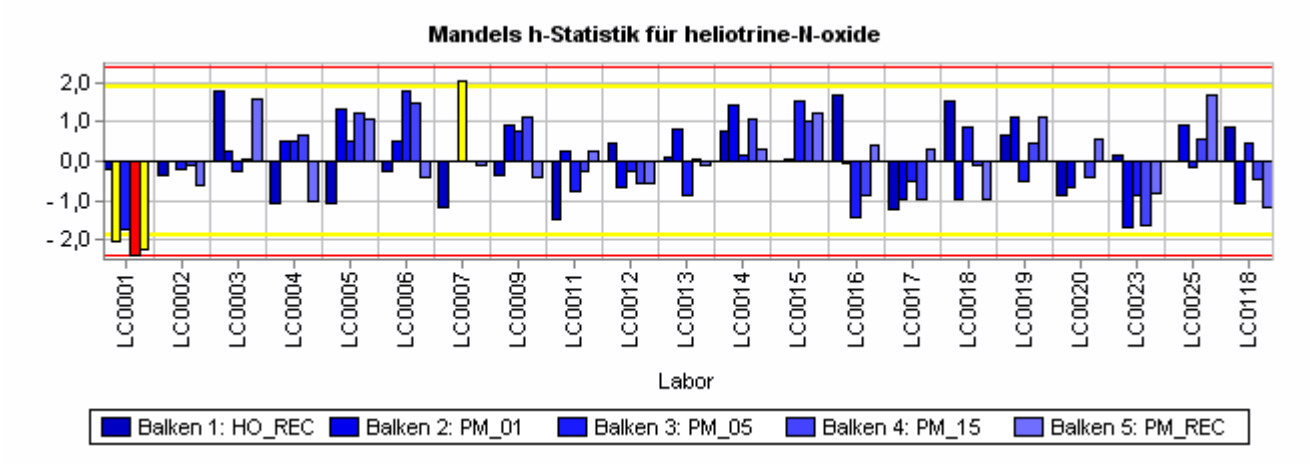
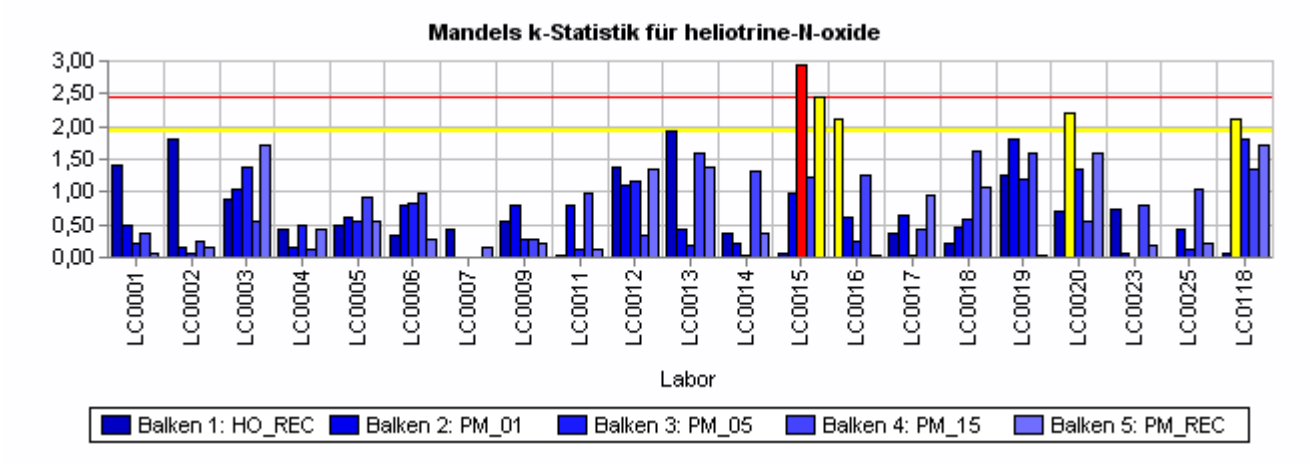
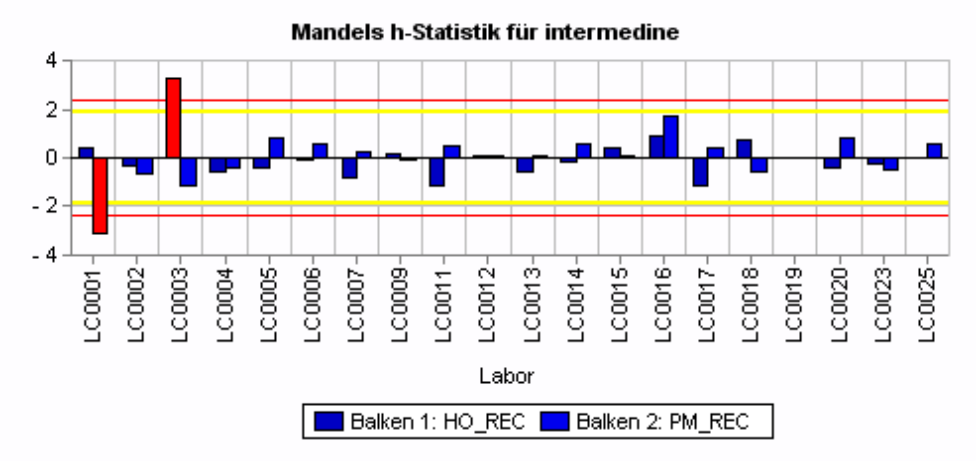
LC0007
LC0009
LC0011
LC0012
LC0013
LC0014
LC0015
LC0016
LC0017
LC0018
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: HO_REC
Balken 2: PM_01
Balken 3: PM_05
Balken 4: PM_15
Balken 5: PM_REC

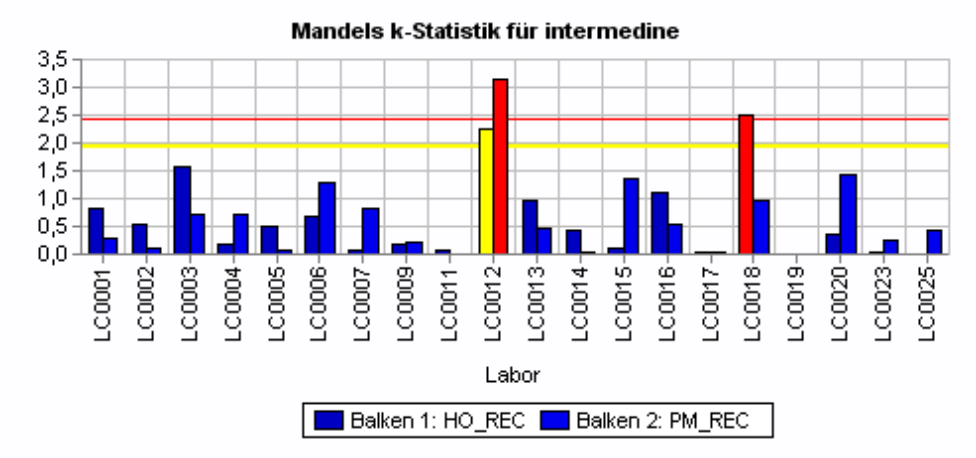
Heliotrine-N-Oxide



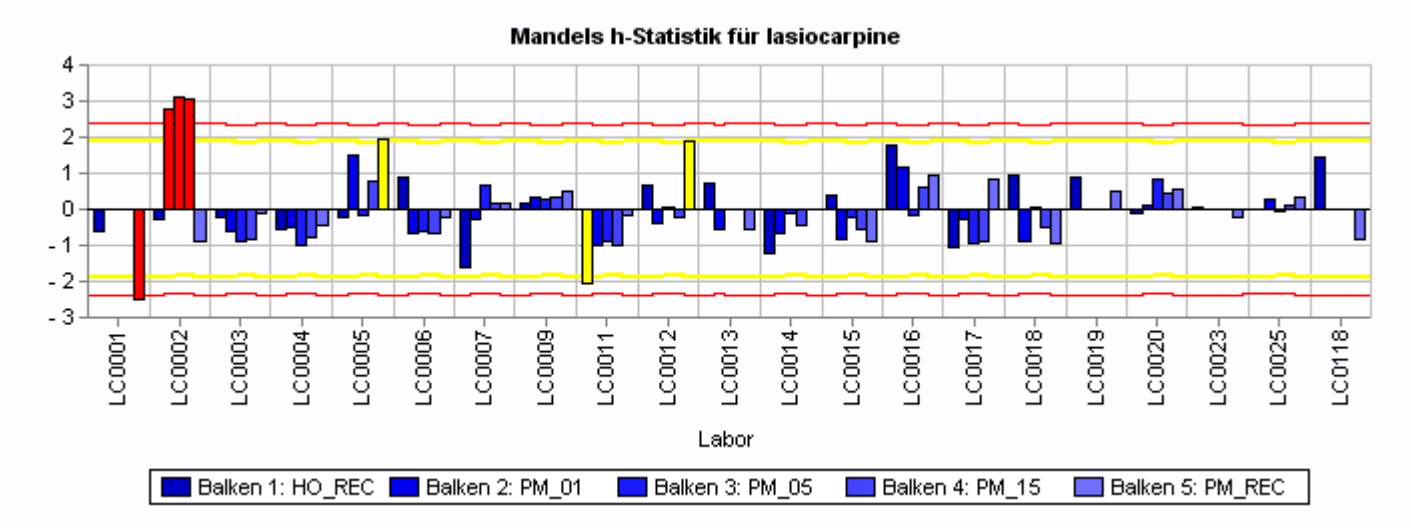


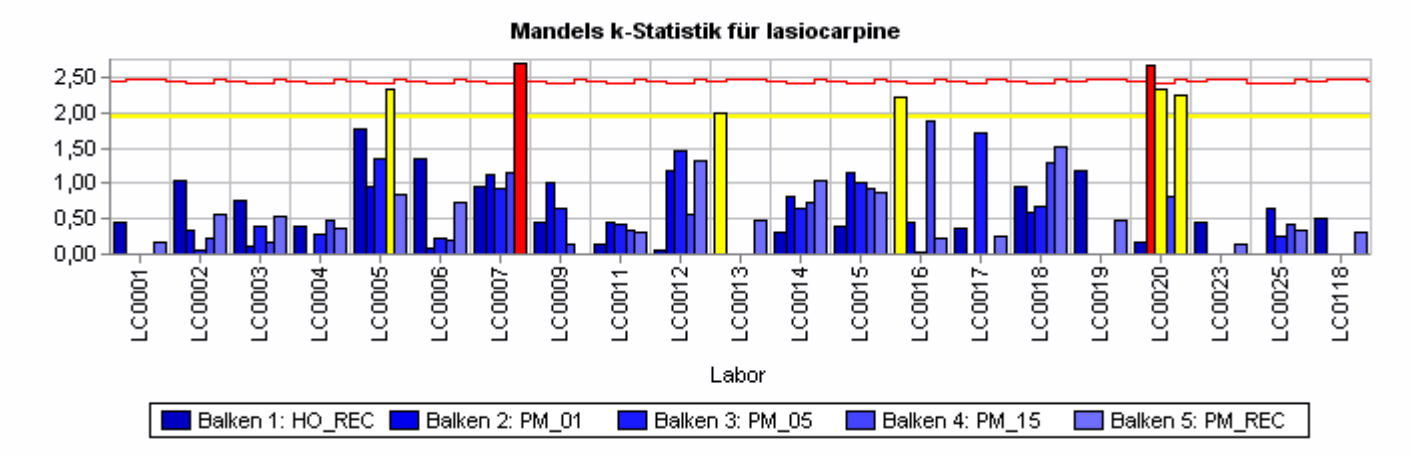
Intermedine



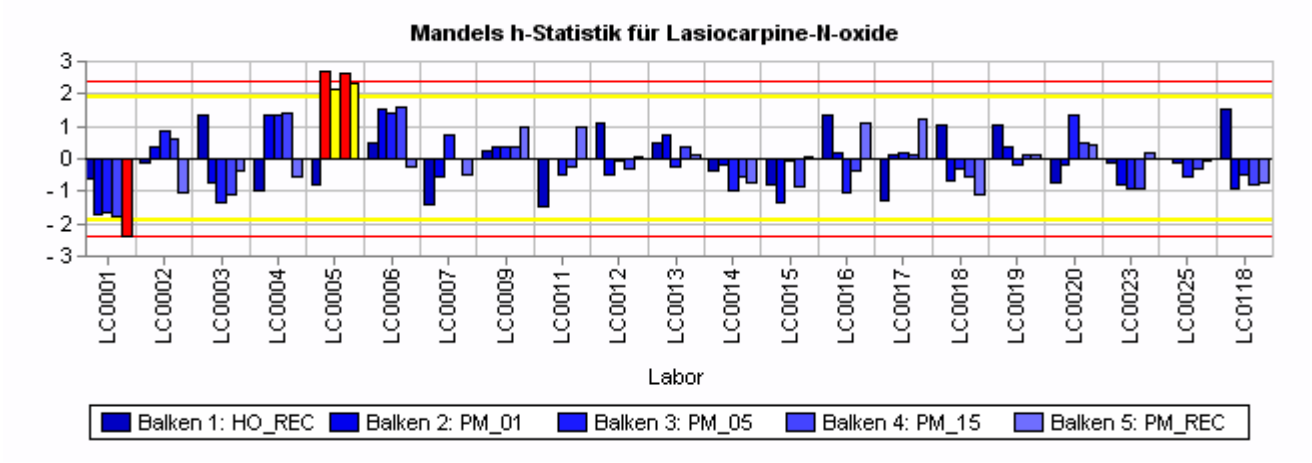


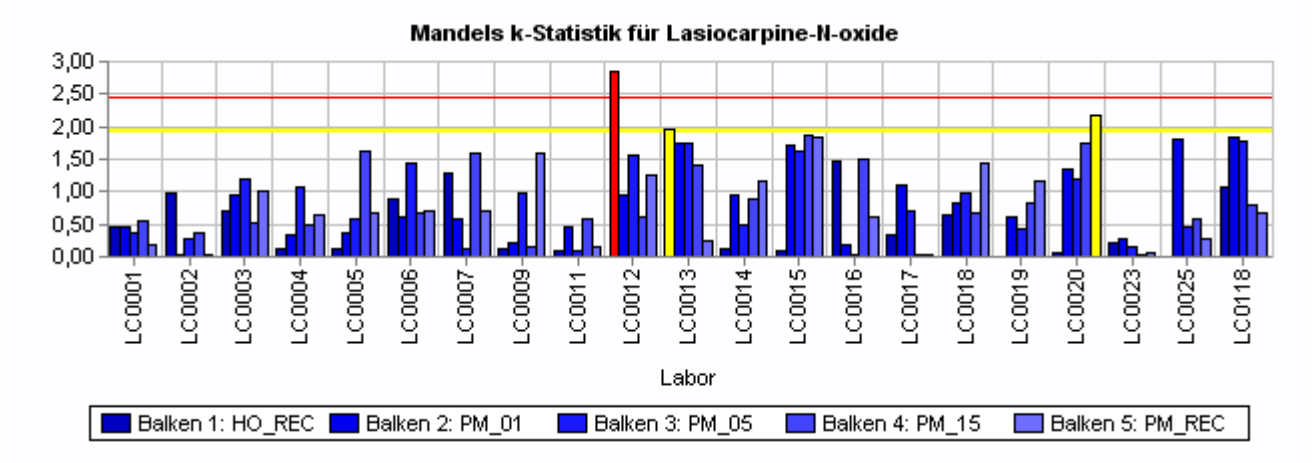
Lasiocarpine



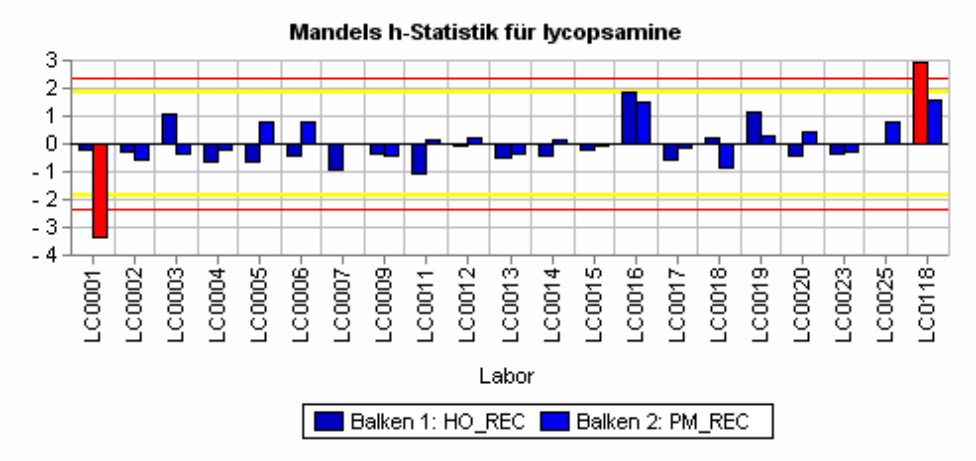


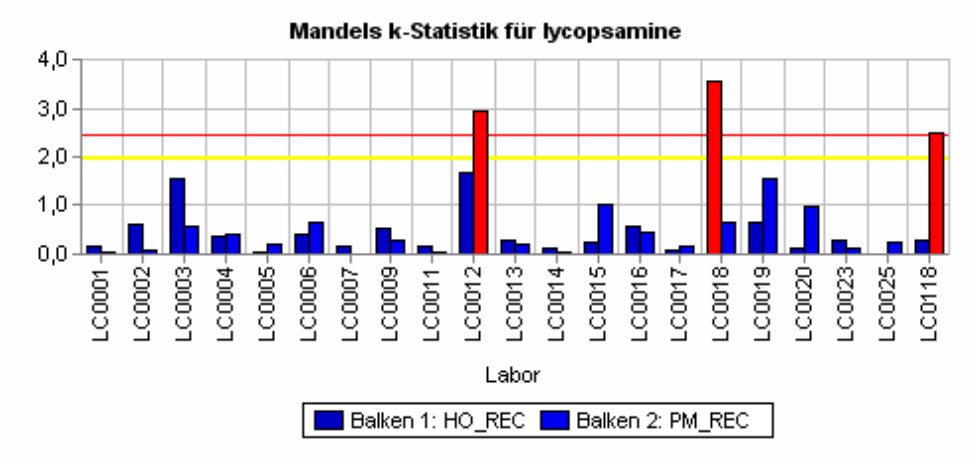
Lasiocarpine-N-Oxid



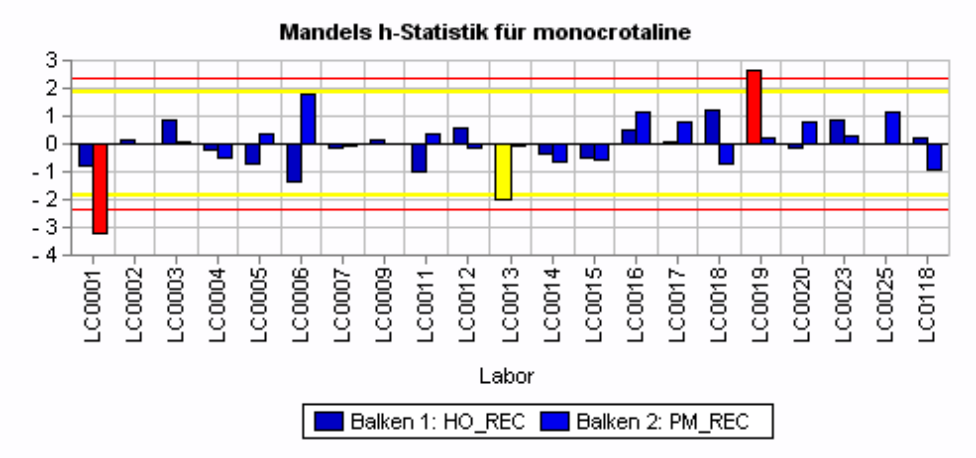


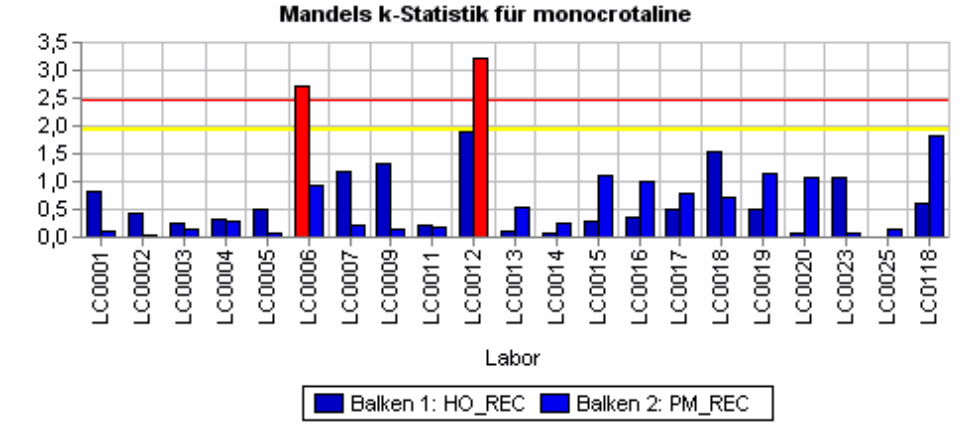
Lycopsamine



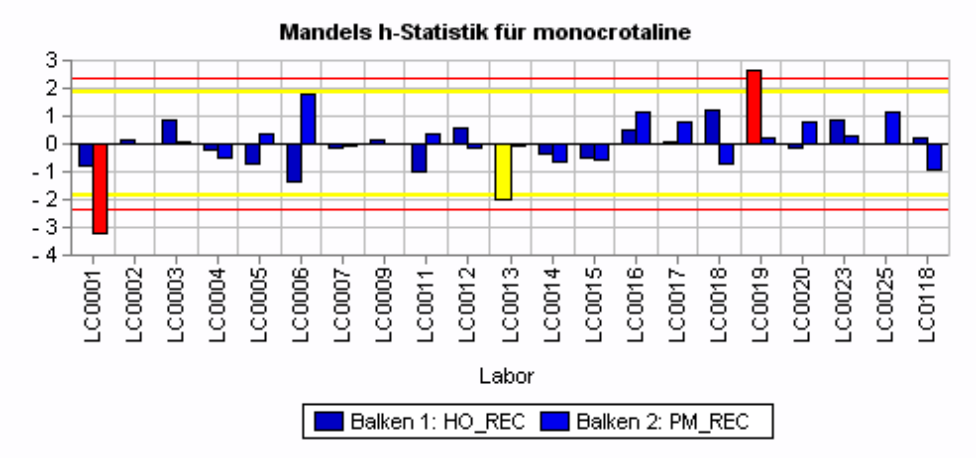


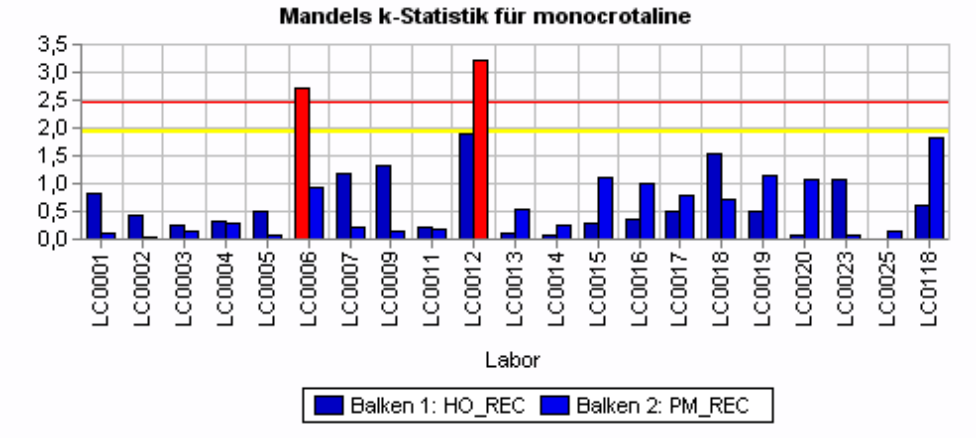
Monocrotaline

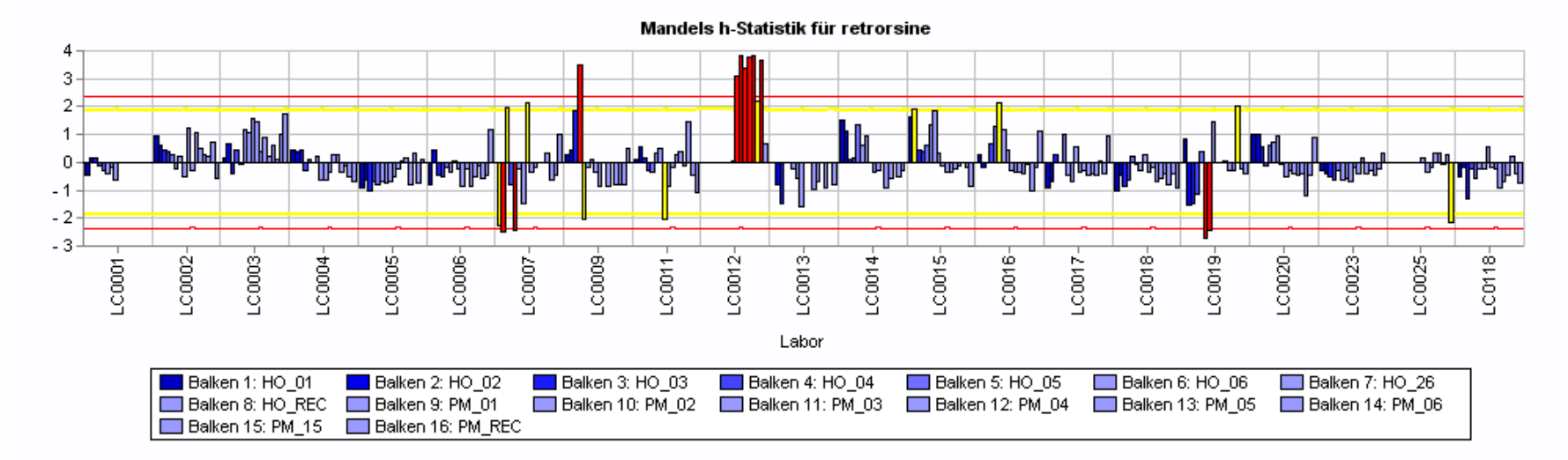




Monocrotaline-N-Oxide



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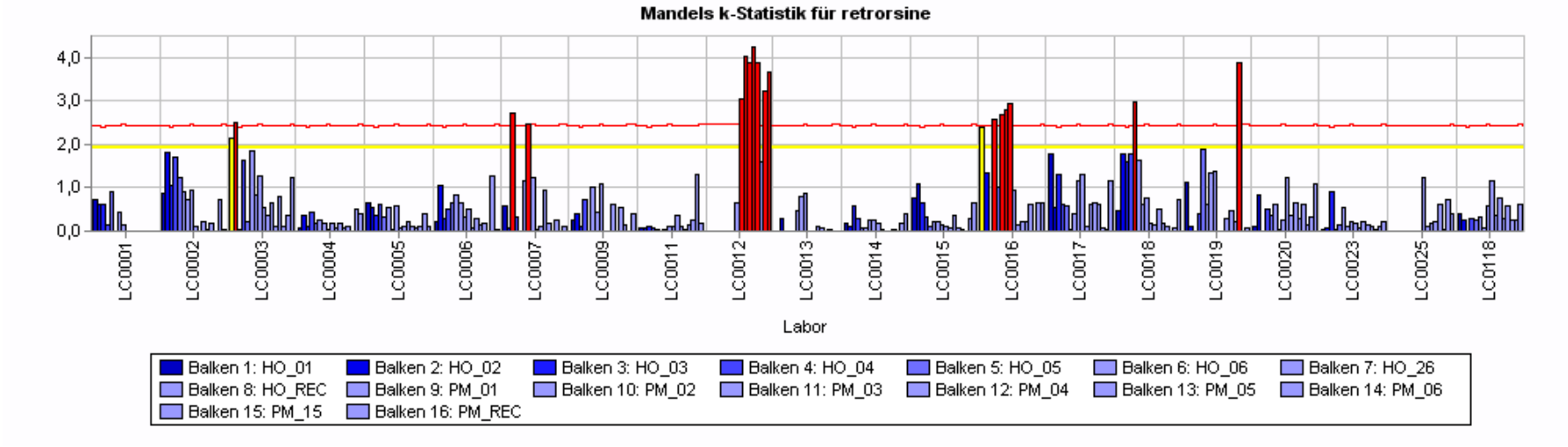
LC0019
LC0020
LC0023
LC0025
LC0118
Labor
Balken 1: HO_01
Balken 2: HO_02
Balken 3: HO_03
Balken 4: HO_04
Balken 5: HO_05
Balken 6: HO_06
Balken 7: HO_26
Balken 8: HO_REC
Balken 9: PM_01
Balken 10: PM_02
Balken 11: PM_03
Balken 12: PM_04
Balken 13: PM_05
Balken 14: PM_06
Balken 15: PM_15
Balken 16: PM_REC


Mandels k-Statistik für retrorsine
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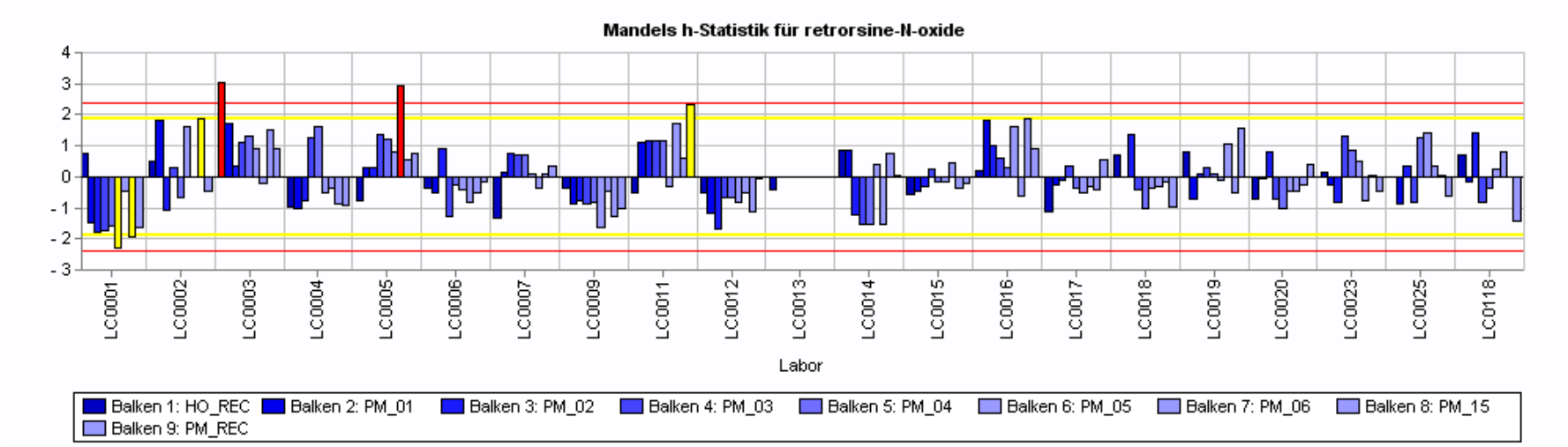
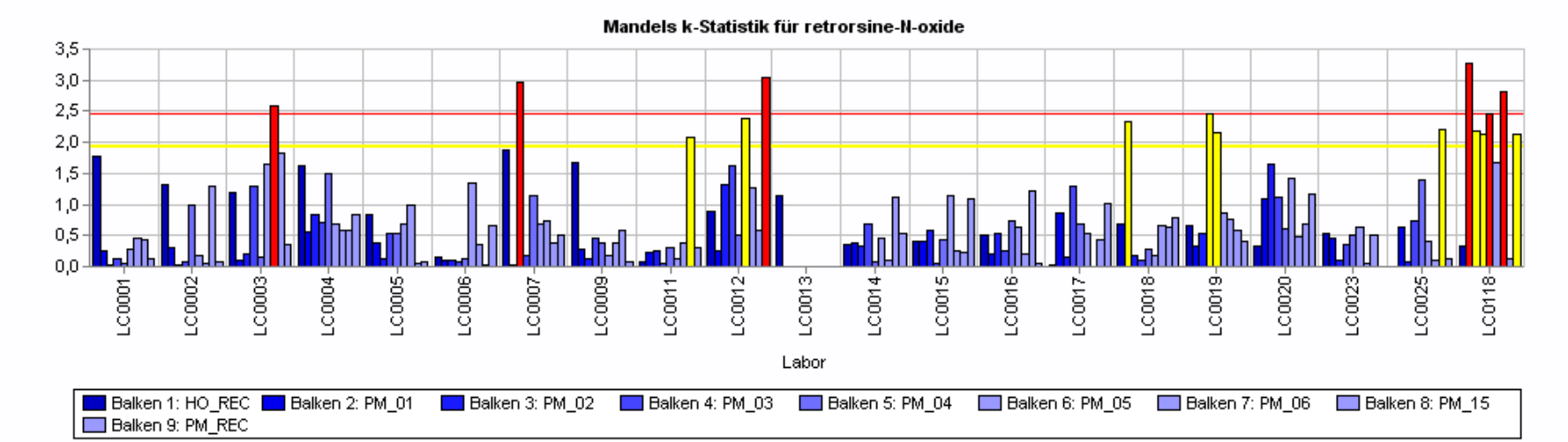
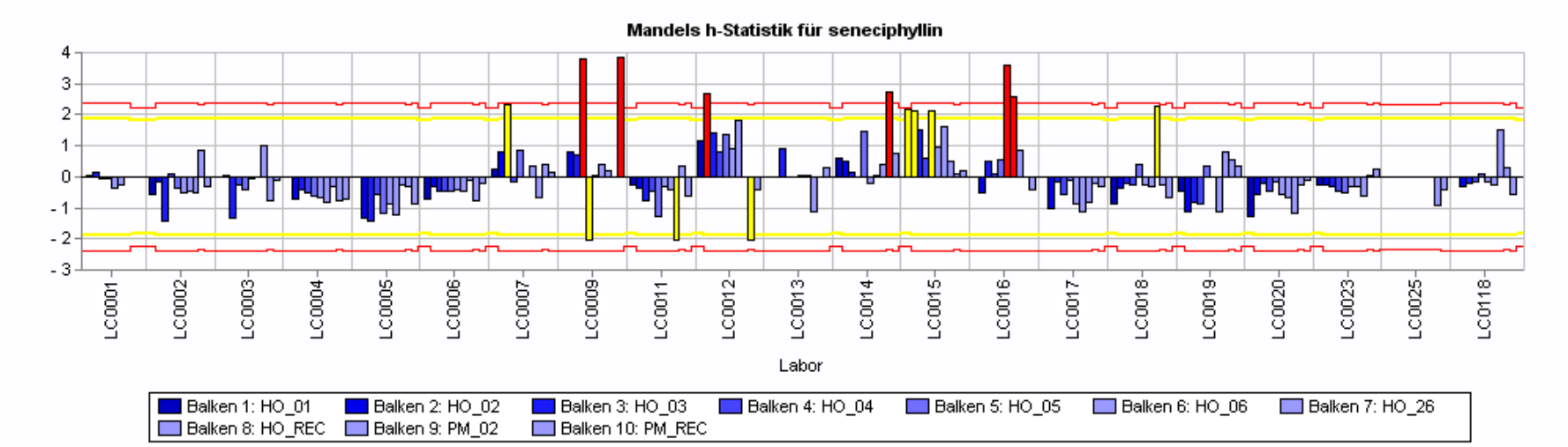
LC0025
LC0118
Labor
Balken 1: HO_01
Balken 2: HO_02
Balken 3: HO_03
Balken 4: HO_04
Balken 5: HO_05
Balken 6: HO_06
Balken 7: HO_26
Balken 8: HO_REC
Balken 9: PM_01
Balken 10: PM_02
Balken 11: PM_03
Balken 12: PM_04
Balken 13: PM_05
Balken 14: PM_06
Balken 15: PM_15
Balken 16: PM_REC

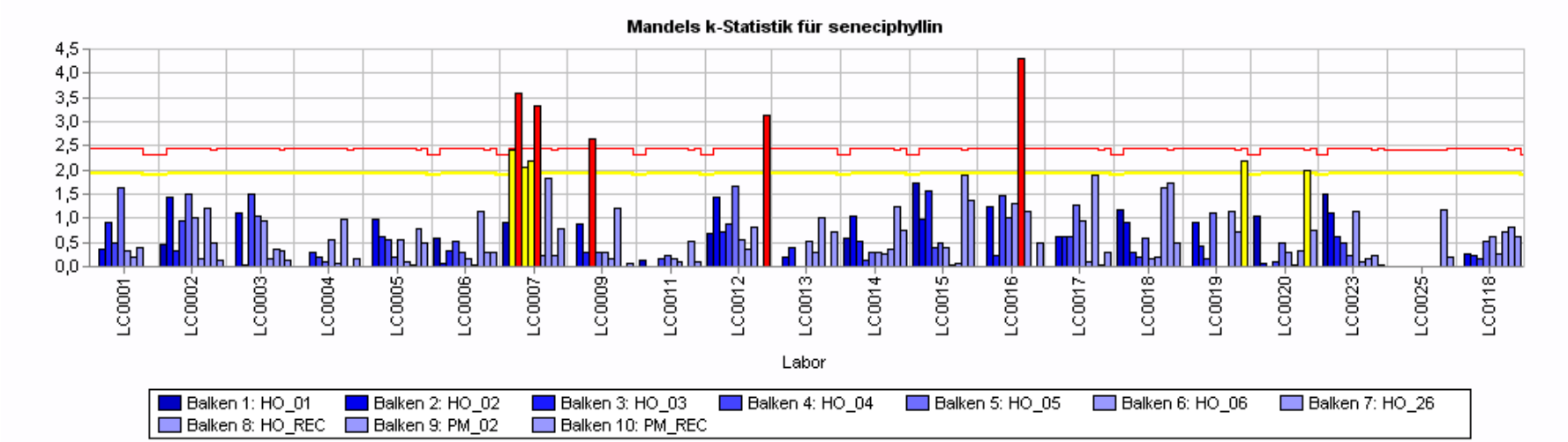
Retrorsine-N-Oxide





Seneciphylline





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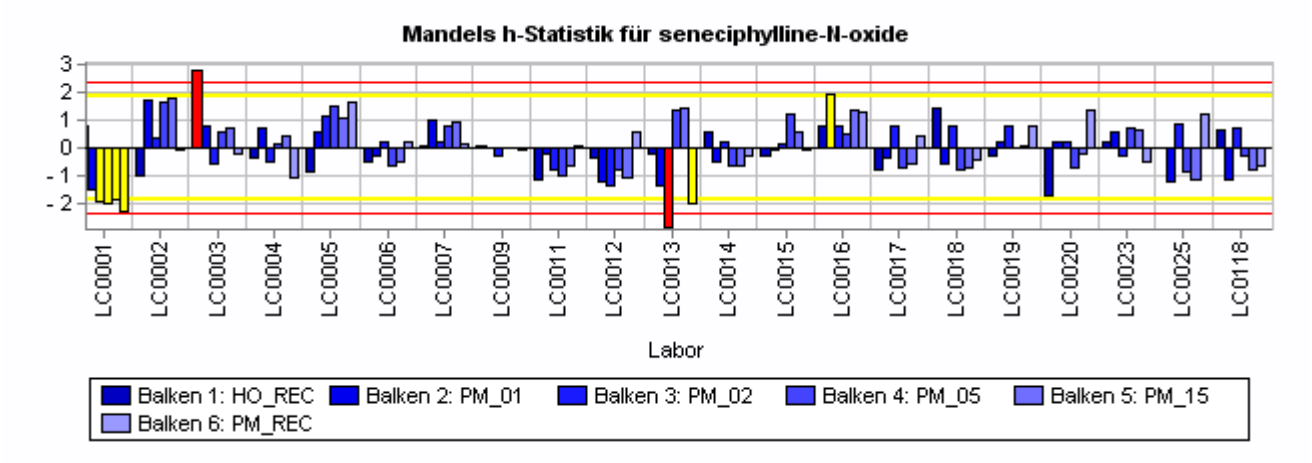

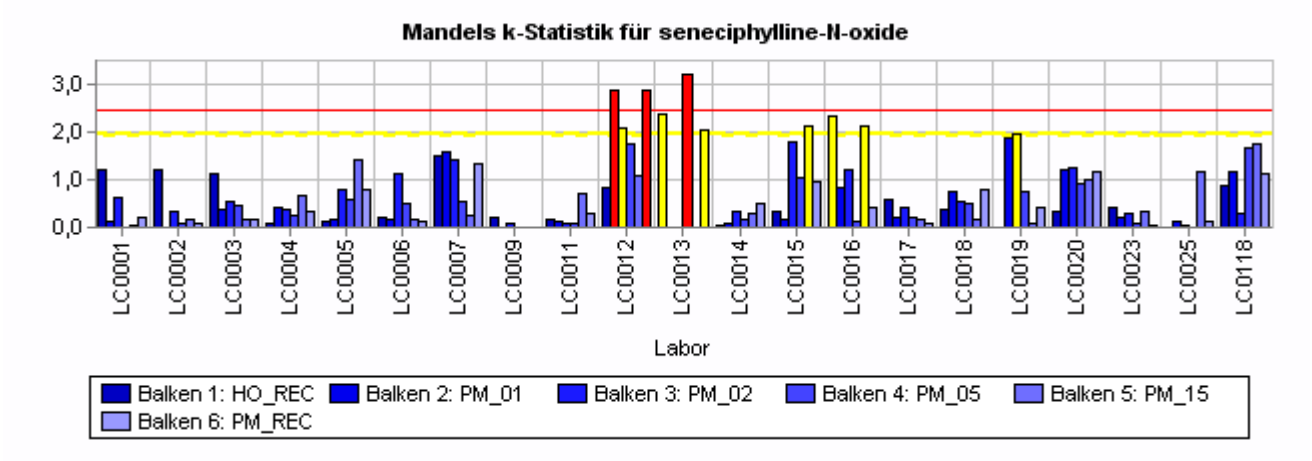

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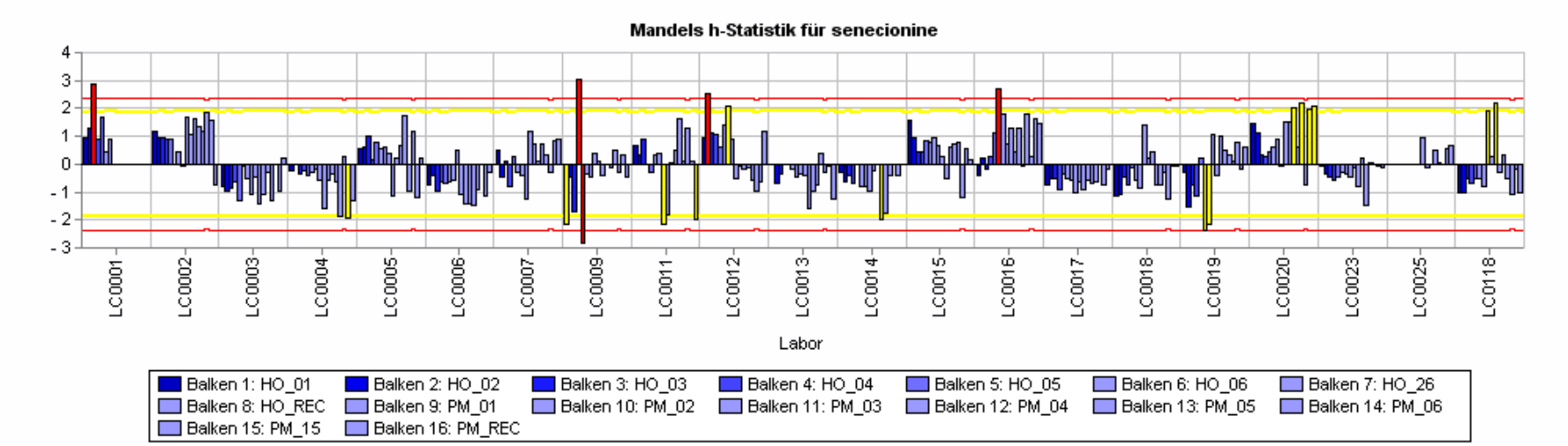

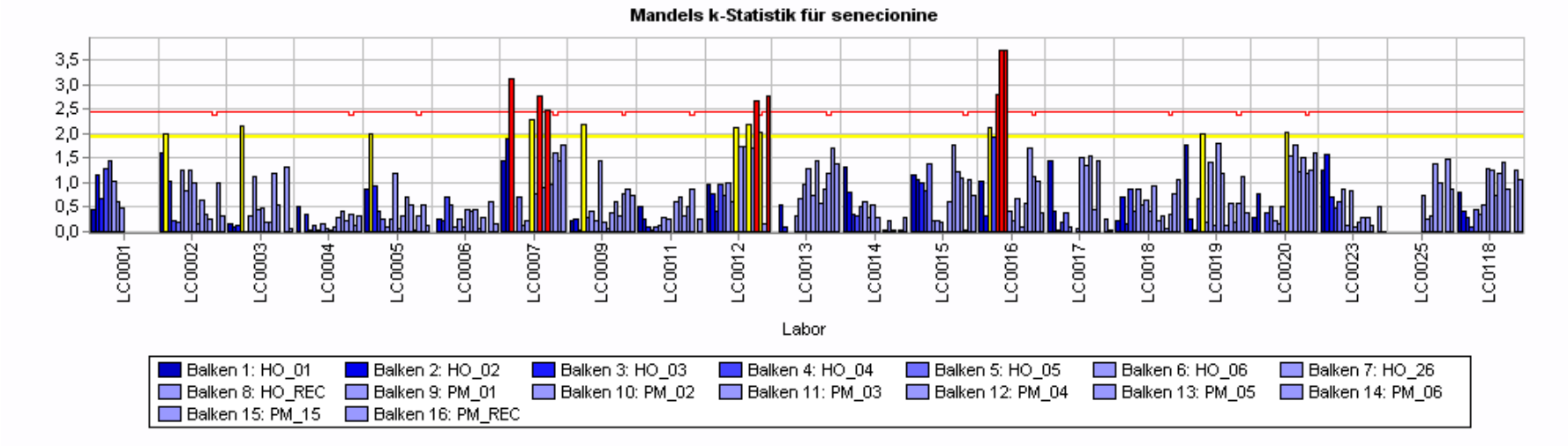

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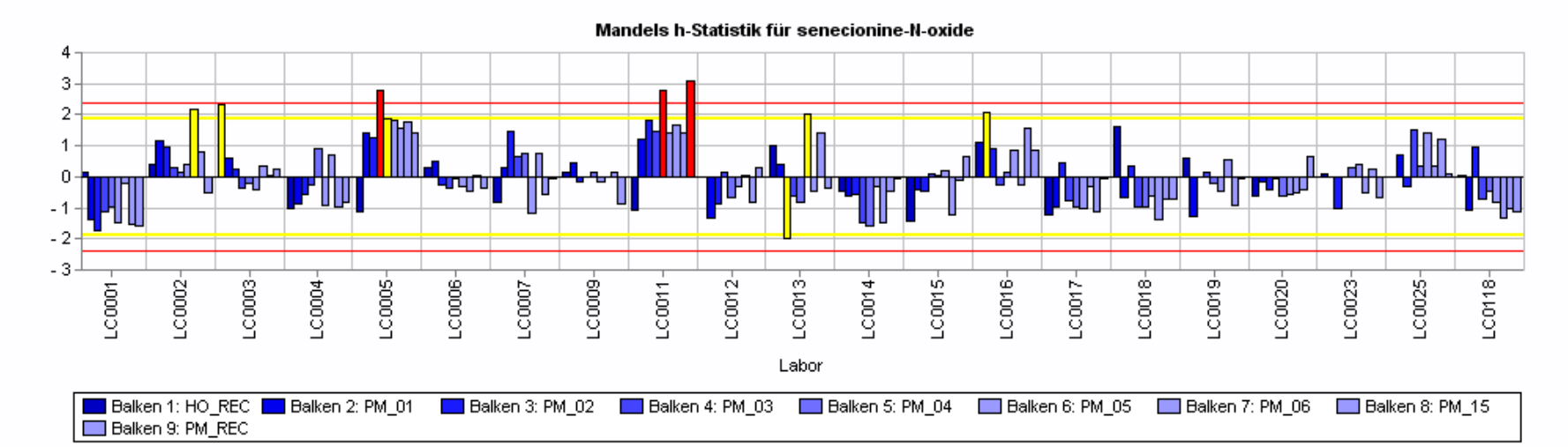

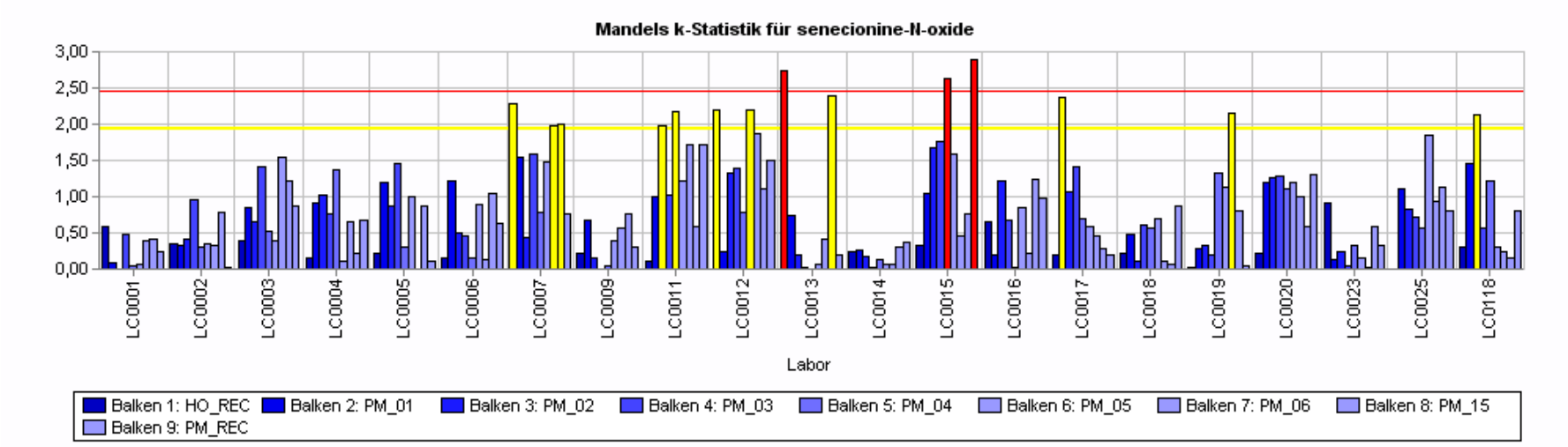

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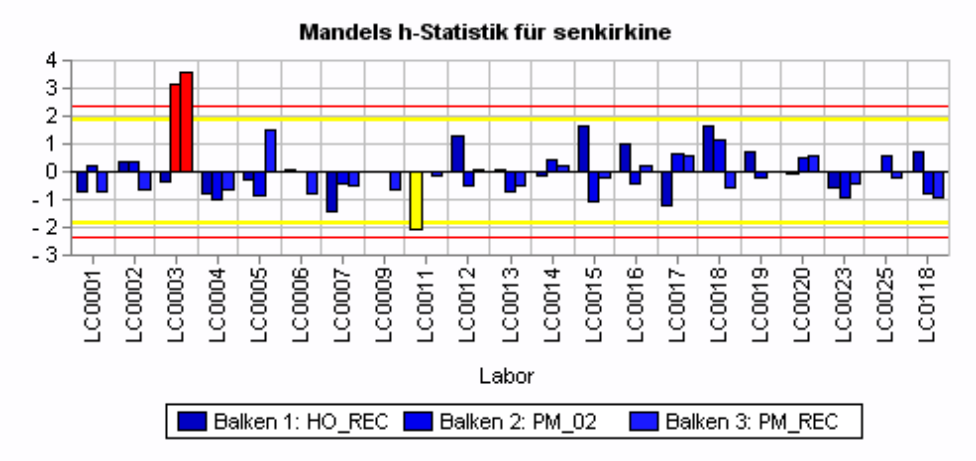
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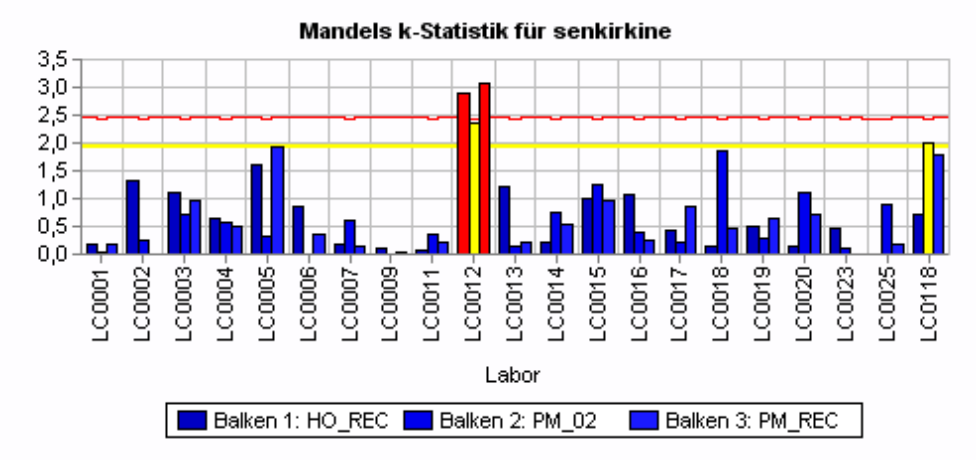
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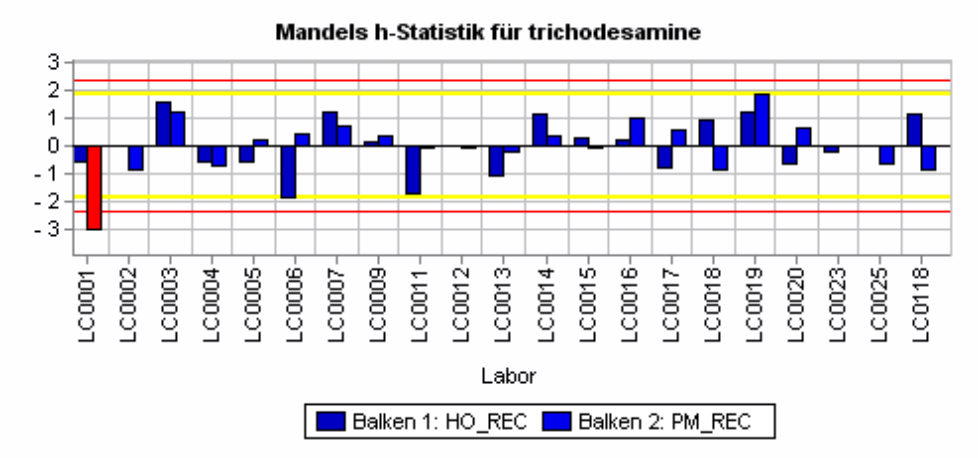
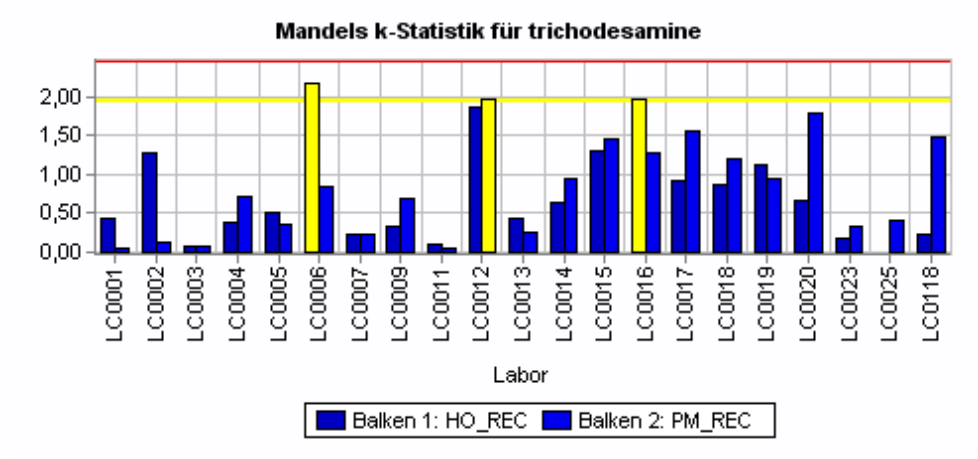
Trichodesmine





5 List of Figures

6 List of Tables