Dioxin analysis : Harmonized Guidelines and Criteria for the Validation and Quality Control

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Introduction

Dioxin analysis
- Qualitative Determination
- Quantitative Determination

Basic Criteria : Complicated Principles
Scientific evidences? Or experiential?

	YES	NO
A Resolving Power ≥10,000 (10%)	O	
B S/N Ratio	O	
C Target Analyte RR CV(%) ≤20	O	
D Labled Analyte RF CV(%) ≤35	O	
E Relative Ion Abundance within ± 15%		O
F Twelve Hour Time Period	O	
G ……	O	
H ……	O	

Analyze Again?

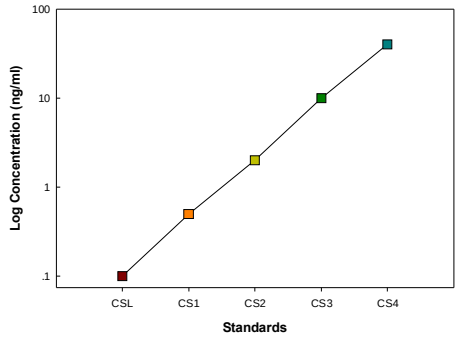
	US EPA Method	United Kingdom
Signal-to-noise ratios	-Qualitative Determination; ≥2.5 for each CDD /CDF detected in a sample extract and ≥10 for all CDDs/CDFs in calibration standard	-Qualitative Determination; ≥2 for all relavent standards >20 for internal quantification standard. -Measured response significantly greater than for blank
Ion abundance ratio	-The ratio of the integrated areas must be within the ±15% of the theoretical ion abundance ratio. -Or within ±10% of the ratio in the midpoint(CS3) calibration or calibration verification(VER).	-Isotope ratio within ±15% of mean for standards
Retention times	-The relative tR of the peak for 2,3,7,8-substituted CDD/CDFs must be within the established criteria limit. -The tR of peaks representing non 2,3,7,8--substituted CDD/CDFs must be within the retention time windows established. -The absolute tR of the $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD GCMS internal standards in the verification test shall be within ±15 s of the tR obtaining during calibration	-Simultaneous (+2/-0s or +2/-0 scans) response for analyte and matching internal standard. -Identical tR (±2s or ±2 scans) for analyte and matching external standard. -For hepta and octa chloro can be increased ±4s or ±4 scans.

	US EPA Method	United Kingdom
Retention times	-The relative tR of CDDs/CDFs and labeled compounds in the verification test shall be within the established limit.	
Recovery	-The recovery of each labeled compound must be within the given limit. If the recovery of any compound fall out side of given limits, method performance is una- cceptable for that compound in the sample. To overcome such difficulties of the performance must be carried out. -Correction : not mentioned	-Correct use of Internal quanti- fication standards(IQS) provides quantitative results wich are auto- matically corrected for recovery and sensitivity validation and should be an integral part of the quantification method. -The use of Internal sensitivity standards is not essential, and the recovery of IQSs can alternatively be assessed by an external standa- rd method.
Smoothing	-Not mentioned	Smoothing process must be investigated as part of initial validation, and the parameters described in the method document and applied consistently
Analytical time limit	-12-hour period of operation is recomme nded	-Not mentioned

Materials & Methods

Sample Preparation

PCDD/DFs standards (CSL,CS1, CS2 and CS3, Wellington Laboratories Inc.)



Quantification and Identification

-High-resolution gas chromatograph (Hewlett Packard 6890 series) coupled with high resolution mass spectrometer (Micromass, Autospec-Ultima).

-Electron impact mode and in the selected ion monitoring mode at a resolution R>10,000 (10% valley) using Masslynx 4.0 program.

-Separation; a DB-5MS (J&W scientific; 0.25 mm ID × 0.25 µm film thickness × 60 m length). The DB-5MS column oven temperature was programmed from an initial temperature of 160℃ to a final temperature of 310℃ (total run time 60 min).

Results & Discussion

1. Ion abundance ratio : major factor?

The changes of ion abundance ratios under the pre- and post- 12 hour period of operation.

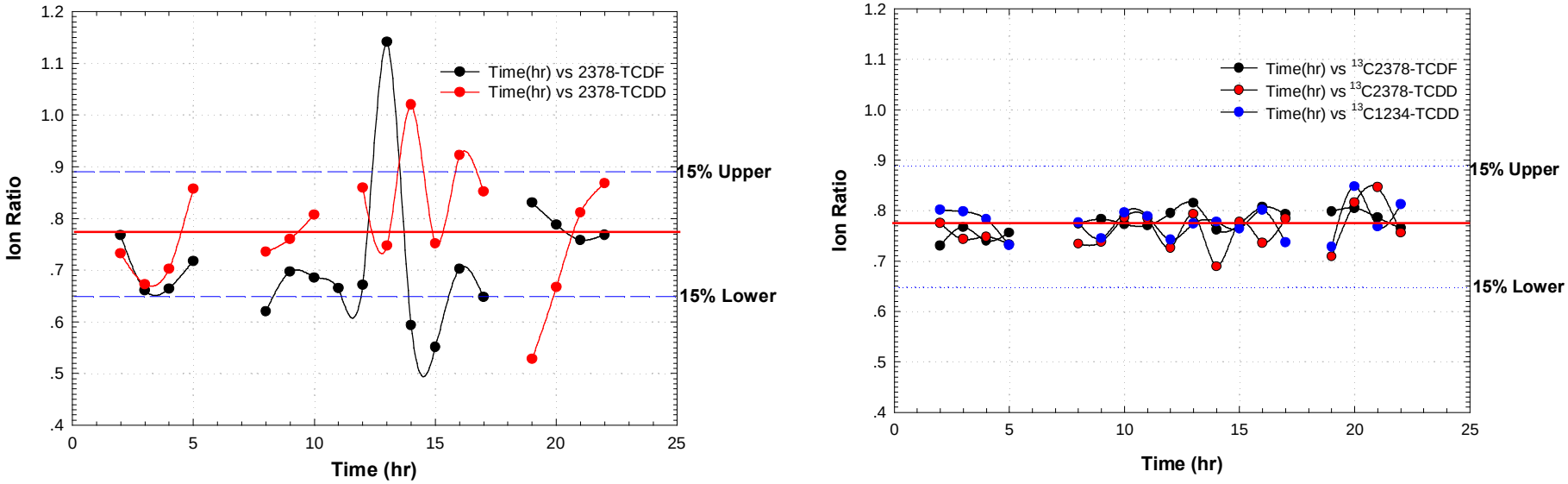


Fig. . The ion ratio change of native and labelled TCDD/F (CS1).

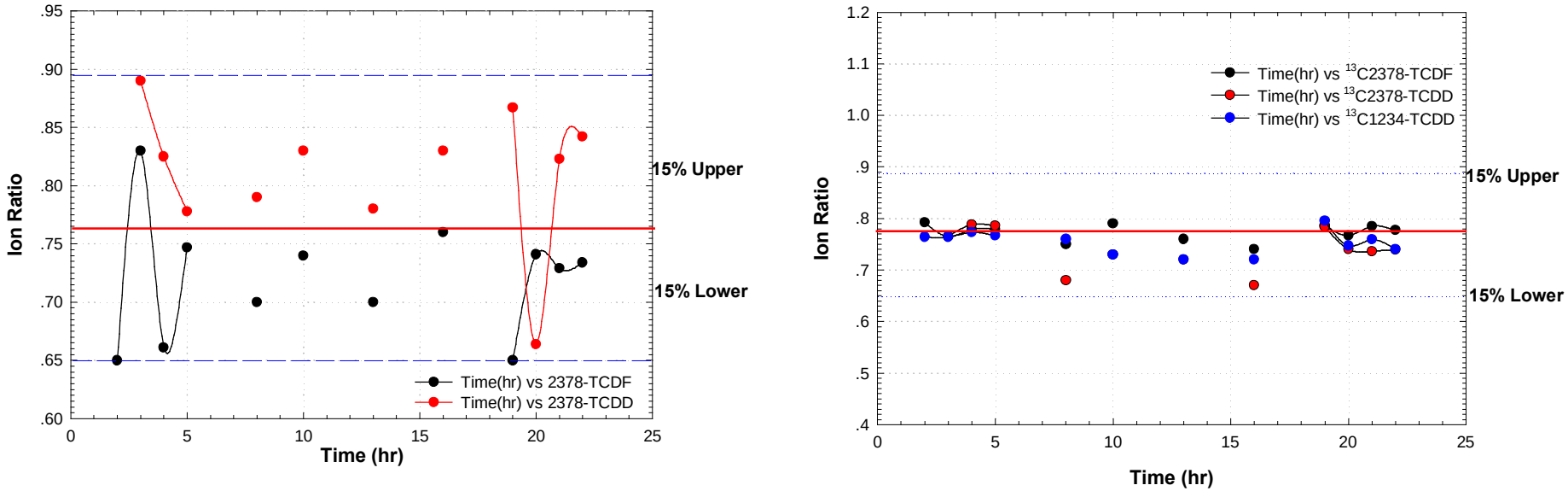


Fig. . The ion ratio change of native and labelled TCDD/F (CS3).

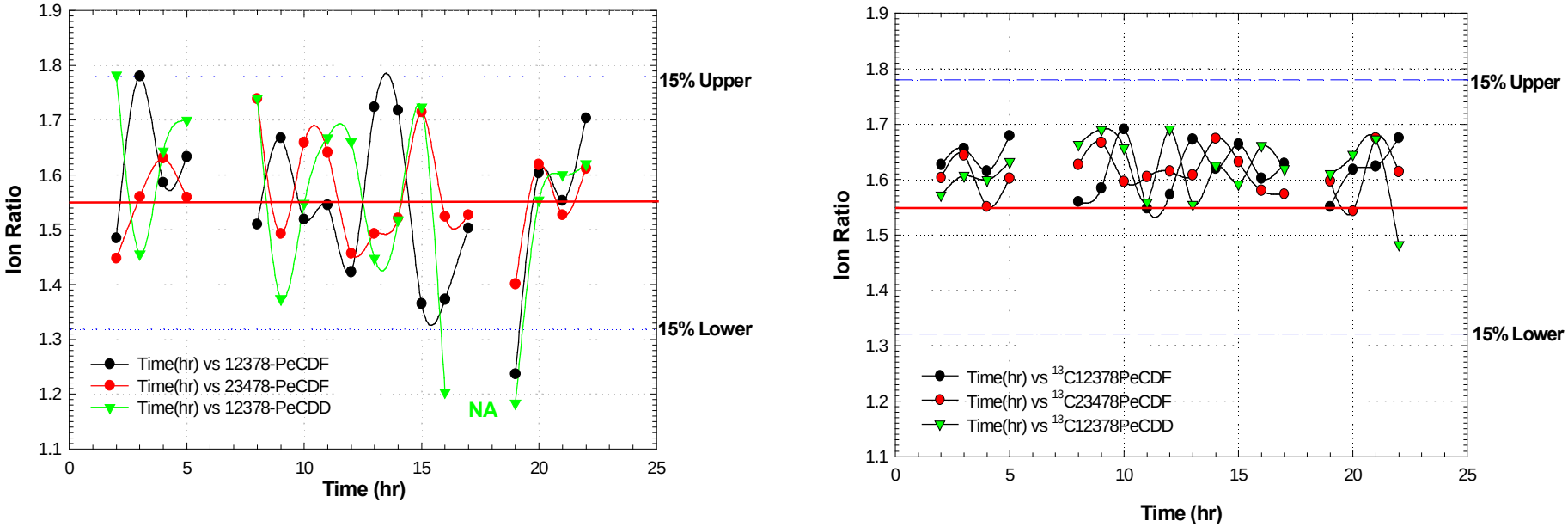


Fig. . The ion ratio change of native and labelled PeCDD/F (CS1).

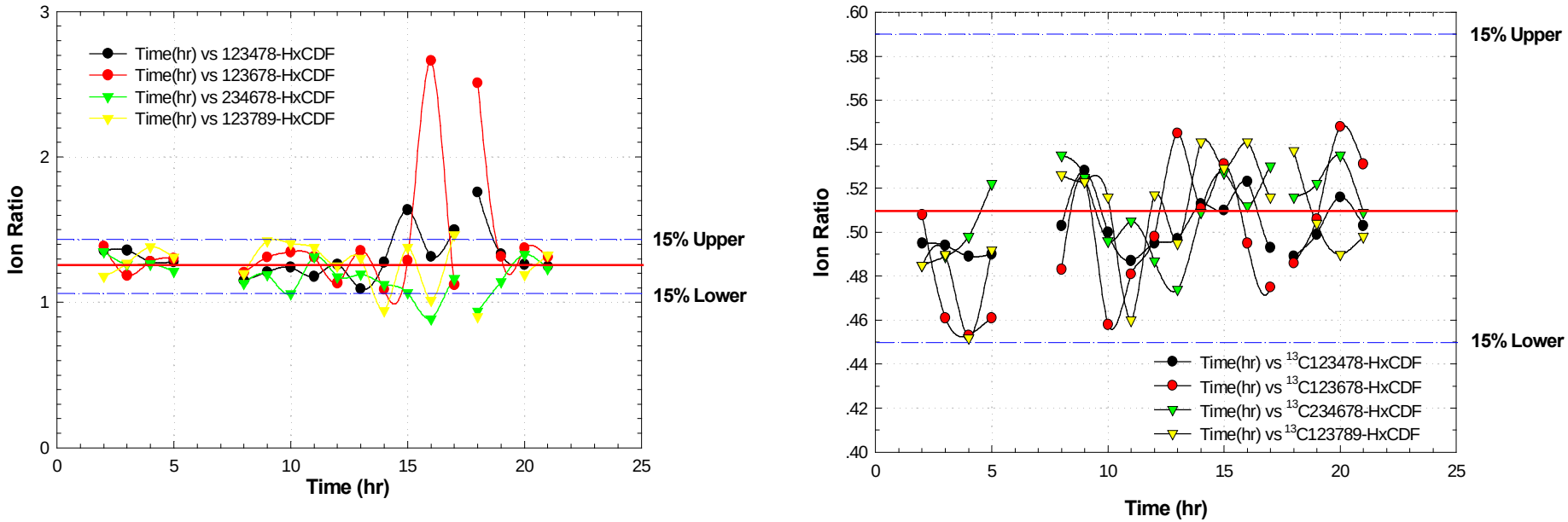


Fig. . The ion ratio change of native and labelled HxCDF (CS1).

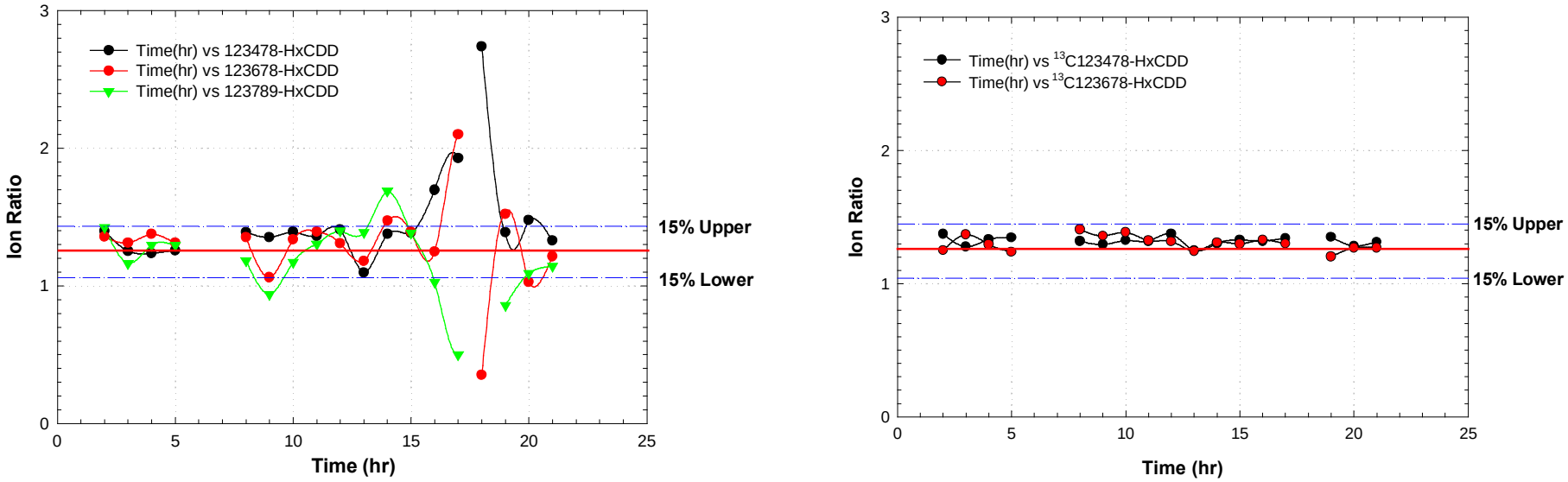


Fig. . The ion ratio change of native and labelled HxCDD (CS1).

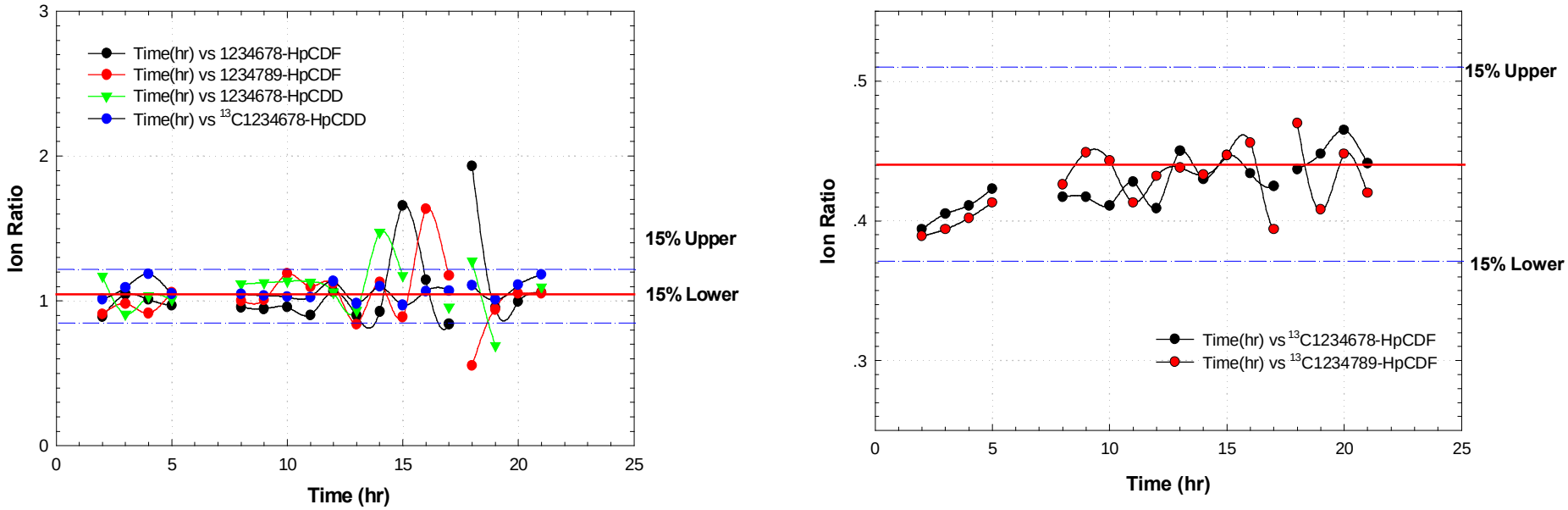


Fig. . The ion ratio change of native and labelled HpCDD/F (CS1).

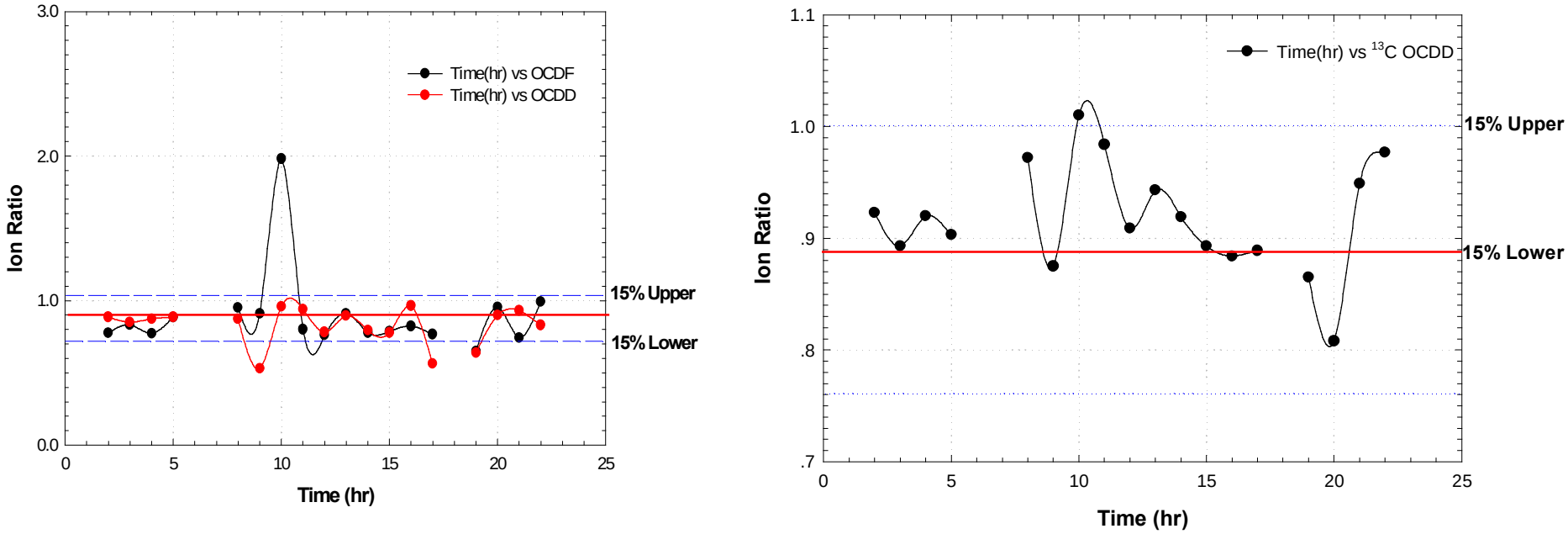


Fig. . The ion ratio change of native and labelled OCDD/F (CS1).

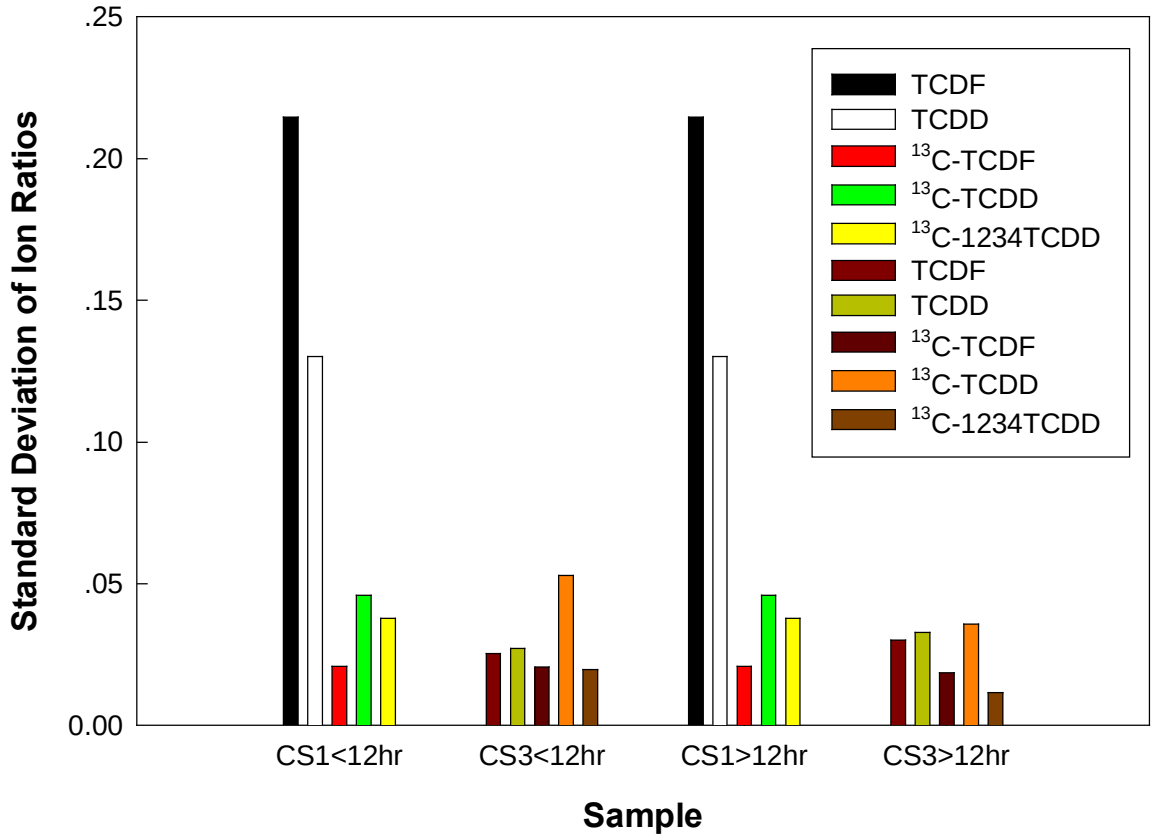


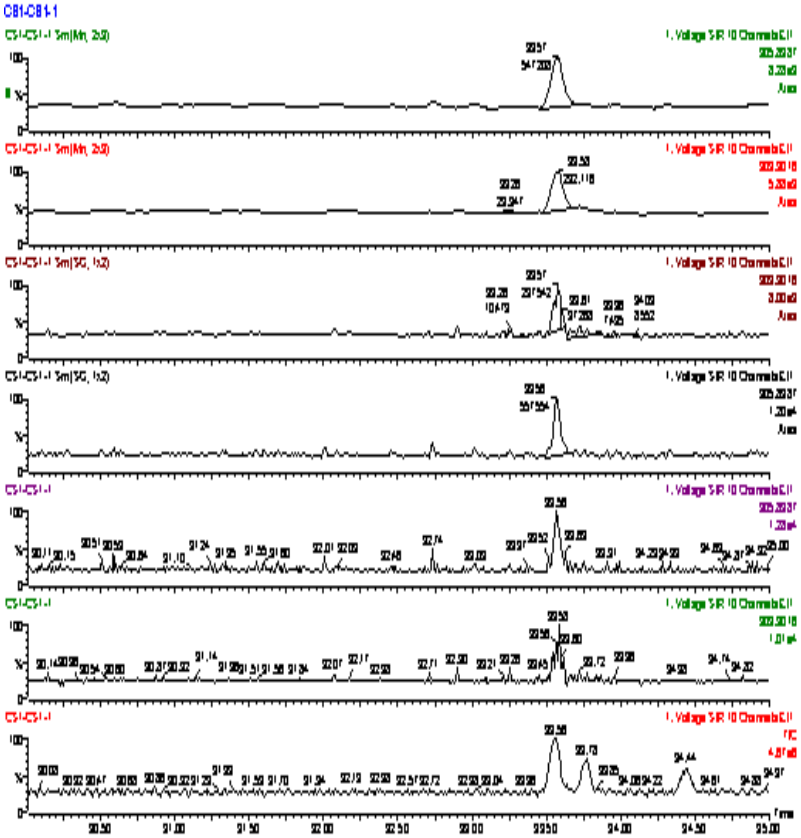
Fig. . The changes of ion abundance ratios between CS1 and CS3 TCDD/Fs under the pre- and post- 12 hour period of operation.

Are there scientific evidences about ion abundance ratios for QC limit? Why analysis must be done within twelve hour time period?

Theoretical Ratio	15% lower	15% higher	20% lower*	20% upper
0.77	0.65	0.89	0.62	0.92
1.55	1.32	1.78	1.24	1.86
1.24	1.05	1.43	0.99	1.49
0.51	0.43	0.59	0.41	0.61
1.05(1.04)	0.89(0.88)	1.21(1.20)	0.84(0.83)	1.26(1.24)
0.44	0.37	0.51	0.35	0.53
0.89	0.76	1.02	0.71	1.07

* ; J.W. Choi, Miyabara Y., Hashimoto S., Morita M. Chemosphere, 2002, 47, 591-597.

2. Integration pitfall by Smoothing



Different results

M/Z	Integration	Area	tR
305.8987	SM(Mn, 2x3)	547.208	33.57
303.9016	SM(Mn, 2x3)	292.116	33.58
303.9016	SM(SG. 1x2)	237.542	33.57
305.8987	SM(SG, 1x2)	557.554	33.56
305.8987	original	n/a	33.56
303.9016	original	n/a	33.58

TIC

Abbreviations:
SM; Smoothing method Mn;mean
SG; Savitzky Golay,
Window size(scans±) x Number of smooths

The registered laboratories should be able to meet the following requirement:

-demonstration of the performance of a method in the range of the tolerance,
***e.g.* 0.5x, 1x, and 2x the tolerance**
-limit of detection should be in the range of about one fifth of the tolerance, to make sure that the acceptable cofficients of variations are met in the range of the tolerance

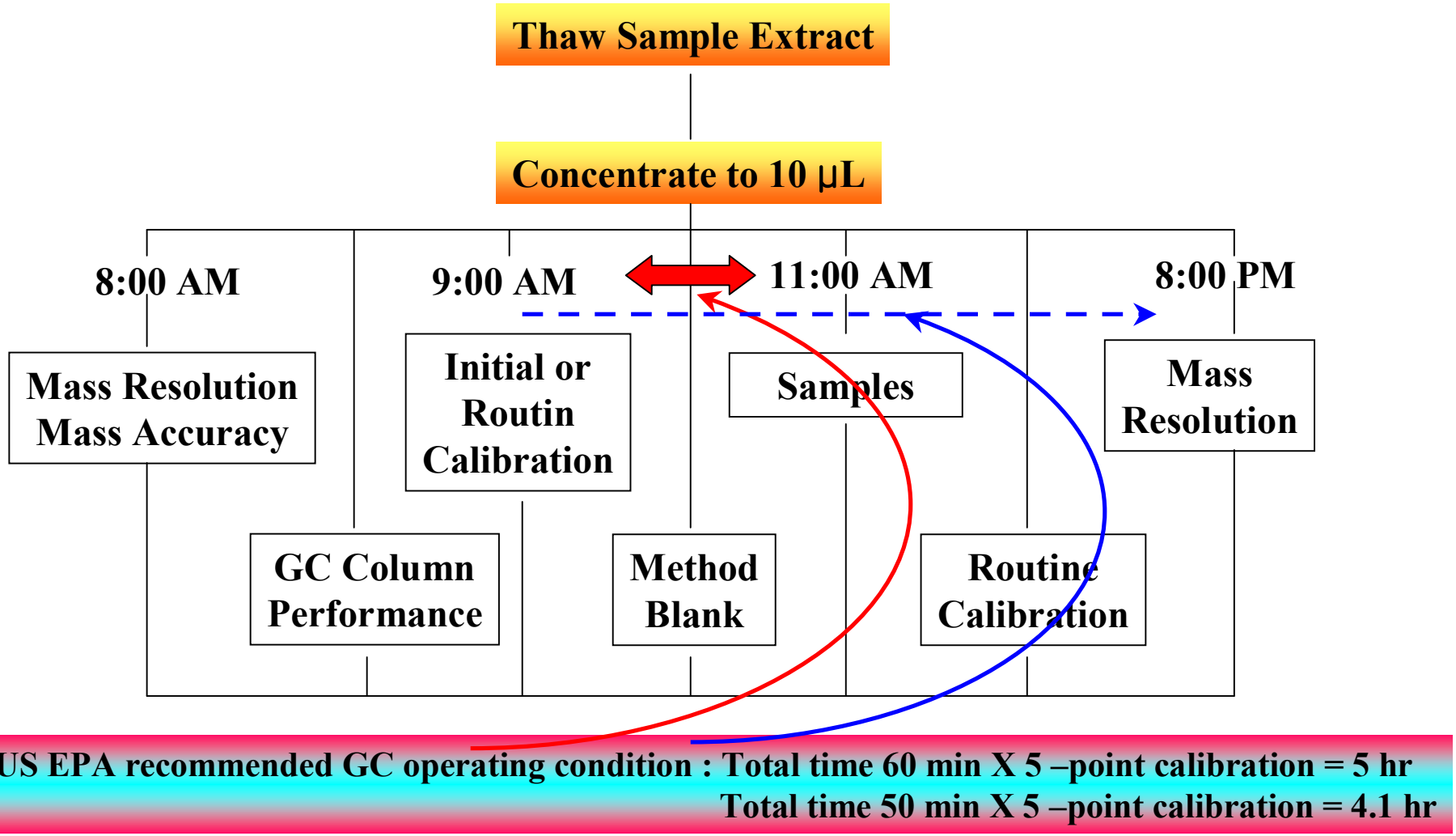
Detection and analysis-Technical considerations

For comparison of analytical results, the limit of detection (lowest limit for qualitative identification, without possibility to quantify the amount) and/or limit of determination (lowest limit for quantification) have to be taken into account. Analytically, all 17 congeners with 2,3,7,8-substitution must be determined. For calculation of the TEQ value, the results of each of these congeners is multiplied by the specific TEF factor and then added up. In most cases, a few of the 17 congeners are below the limit of detection and/or limit of determination. This can become critical if many congeners are not determinable or if the toxicological most important congeners are not found. Some laboratories are used to calculate the contribution of not detectable congeners to the TEQ as "0". As a consequence, low dioxin contents could have been the result of really low levels of the sample or of insufficient detection/determination limits, without considering these detection/determination limits in the final TEQ calculation. To make sure that low dioxin levels are really the result of low levels in the sample, the concept of tolerances "as upper bound limit of detection" or "upper bound limit of determination" was developed. This concept demands the inclusion of the full limit of detection or determination instead of "zero" for not detectable substances. It should be applied generally, with a clear preference of "upper bound limit of determination" rather than upper bound limits of detection.

For montmorillonite/bentonite, a laboratory has found < 1.9ng I-TEQ/kg. Thus, obviously dioxins were not detectable with a limit of determination of 1.9ng I-TEQ/kg. For the same group, a laboratory of the same country (maybe the same laboratory?) found 1.7ng I-TEQ/kg. It remains unclear whether the range of 1.5 to 2ng I-TEQ/kg was the practical limit of determination of that laboratory. This may be acceptable in a crisis situation (*e.g.* after the first finding of the contamination of kaolinitic clay) to see whether there are other highly contaminated samples, as well. However, these values cannot be used for definition of the background contamination, as the applied method is obviously not suitable for this purpose. Moreover, the method is not suitable for determinations in the range of the tolerance of 0.5 ng WHO-TEQ/kg which was set in response to the finding of this contamination.

European Commission (2000), Directorate C –Scientific Opinions, Opinion of the scientific committee on animal nutrition on the dioxin contamination of feedingstuffs and their contribution to the contamination of food of animal origin.

Practically, How Many Samples Can You Analyze within 12 hours ?



Cited ; US EPA, 1998, Method 8290A

Conclusion

Uncertainty factors in qualitative/quantitative determination and QC for Dioxin analysis

US EPA methods or others methods → **-obscure expression, sometimes just as guideline**
-something are not practical in a Lab.
-may<must be followed, but able to modify

✓ **Integration** **-Instrumental Detection Limit(IDL) is limited by customized standard solution**

S/N ratio of IDL >10 (in a real experiment)

Criteria of S/N ratio for qualitative determination ; >2.5

Quantitation **GAP** **Qualification**

-Different integration technique makes different results
(researcher's arbitrary integration and different operating programs)

✓ **Stability of instrument** **Isotope Dilution Method calculation**

↓

-Data of Internal standards & Labeled compound after 12 time period OK

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Calculation No Problem ← **?**

Proposal and References

- **For Standardization and Harmonization of Dioxin Analysis, Integral QC/Performance Criteria Are Necessary Between Inter/Intra Organization of Government and Research Laboratories.**

- **References**

1. **US EPA (1994) Method 1613B, Tetra through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS, Office of Water, Washington, D.C.**
2. **US EPA (1998) Method 8290A, Polyclorinated dibenzodioxins and polychlorinated dibenzofurans by HRGC/HRMS, Office of Water, Washington, D.C.**
3. **US EPA (1997) 40CFR Part 136 [FRL-5889-3], Guidelines establishing test procedures for the analysis of pollutants; EPA Method 1613; Final Rule.**
4. **Ambidge, P.F. et al. (1990) Chemosphere. 21,8, 999-1006**