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Estimating Confidence Intervals for Metabolite Degradation Rates

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Outline

- Introduction
- Approaches to Estimating Confidence Intervals
- Examples
- Conclusions

Introduction

- Kinetic analysis to determine degradation rates of parent compounds and metabolites for EU registration is performed according to the report of the FOCUS Work Group on Degradation Kinetics (2006).

- The FOCUS recommendation is that degradation rates for metabolites should preferentially be derived with studies starting with parent.
  - Advantage of deriving formation fraction in the same experiment.

Introduction

- If the confidence interval of the metabolite degradation rate includes zero, then the FOCUS approach cannot determine whether degradation is occurring, even when the observed data exhibit a decline.
  - In these cases, a conservative decline rate or default half-life of 1000 days can be imposed.

- When degradation rates of metabolites in parent applied studies are not significantly different from zero, additional degradation studies starting with the metabolite are often performed.
  - Preferable to derive parent and metabolite kinetics from the same study.

Introduction

- As noted in the FOCUS kinetics work group, confidence intervals from nonlinear least squares (NLS) analysis can be too wide.

- This work has focused on implementation of standard and well-established statistical techniques to more accurately represent confidence intervals.

Conventional Approach

- The NLS method makes the following assumptions for determining confidence intervals:
  - Error variance is the same for all data (for example, levels of parent and metabolites).
  - The errors are normally distributed around zero.

- When used for parent-metabolite kinetic analyses, NLS provides conservative estimates of metabolite confidence intervals if the error variance of parent is larger than for the metabolite.
  - This could occur, for example, when metabolite levels are low compared to parent.

Alternative Approaches

- Two standard approaches can provide more refined estimates of confidence intervals:
  - MCMC (Markov Chain Monte Carlo) analysis
  - IRLS (Iteratively Reweighted Least Squares) routines

- These approaches do not require the assumption that error variance is the same for parent and metabolite.

Alternative Approaches

- No additional information is required to apply these techniques to standard data sets.
- Use of these techniques conforms with the guidance in the FOCUS kinetics report.
- Focus of this work has been to determine the effect of using of these techniques on:
  - Values of resulting kinetic parameters
  - Confidence intervals

Markov Chain Monte Carlo

- MCMC is a stochastic method that explores a given distribution of model parameter values.
- The Markov Chain is a chain of parameter sets in which each element is generated from the previous one by a jump function that contains a random component.
- Generates actual distributions for both parent and metabolite parameters which can be used to determine the confidence intervals.

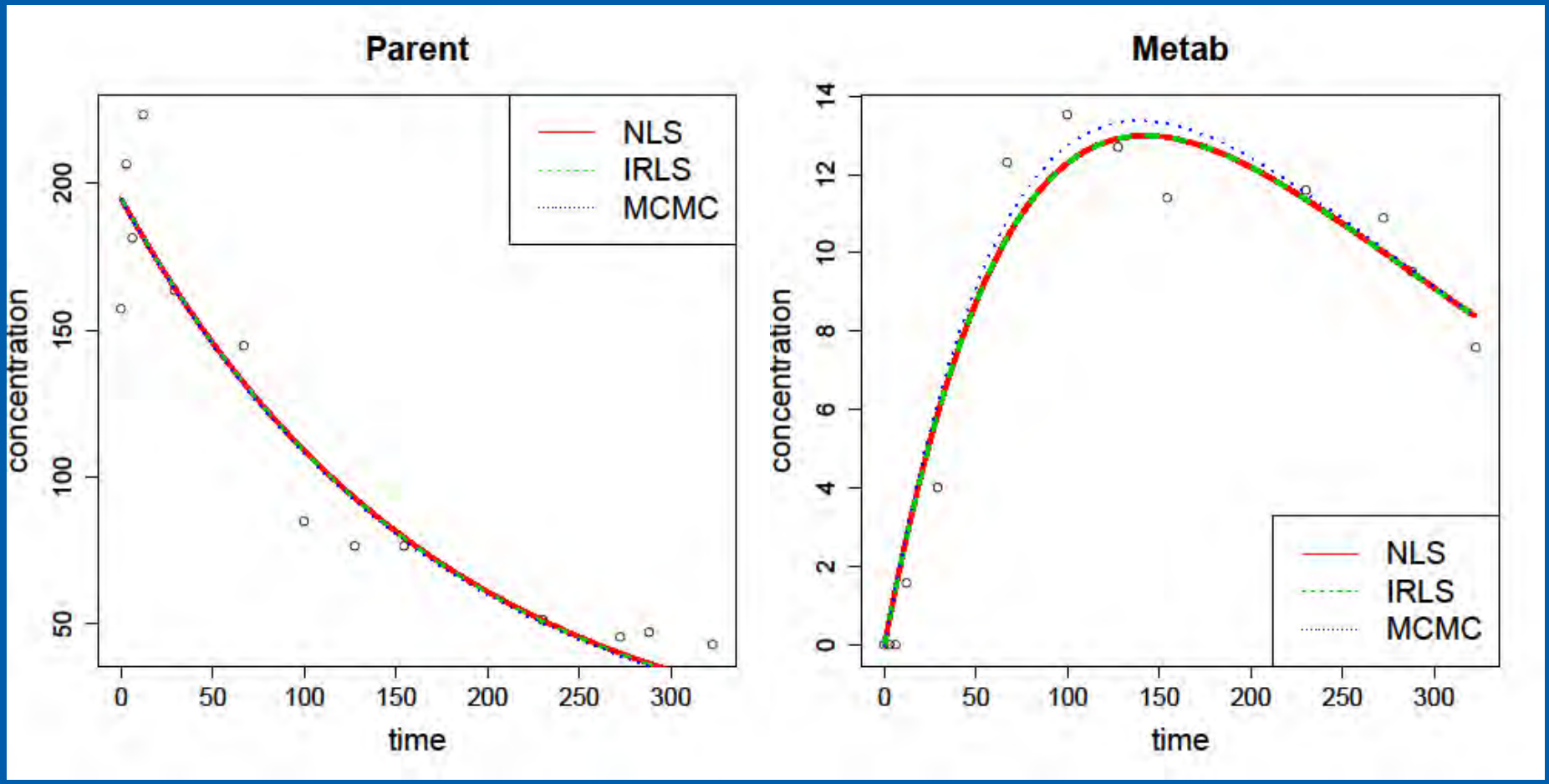
Iteratively Reweighted Least Squares

- IRLS is similar to NLS, except that the variance of the errors of parent and metabolite can be different.
- The observations are weighted according to the error variance.
- Then the regression process is repeated and the weights adjusted according to the new error variance, until convergence is achieved.

Testing Experiences

- A number of data sets have been tested with both the IRLS and MCMC methods.
  - Generally give about the same solution and confidence intervals.
  - Narrower metabolite confidence intervals than NLS when error variance for parent is greater than for the metabolite.
  - In cases when degradation is not occurring, both methods generate confidence intervals that do contain zero.

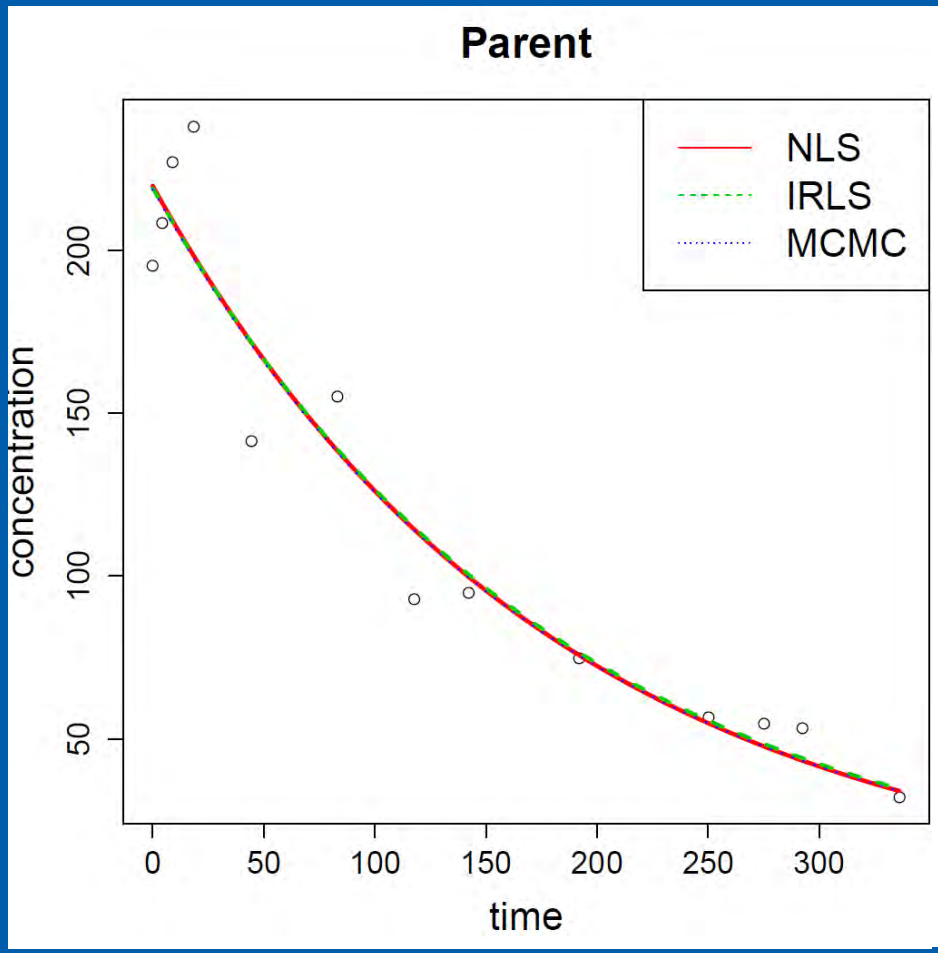
Example 1

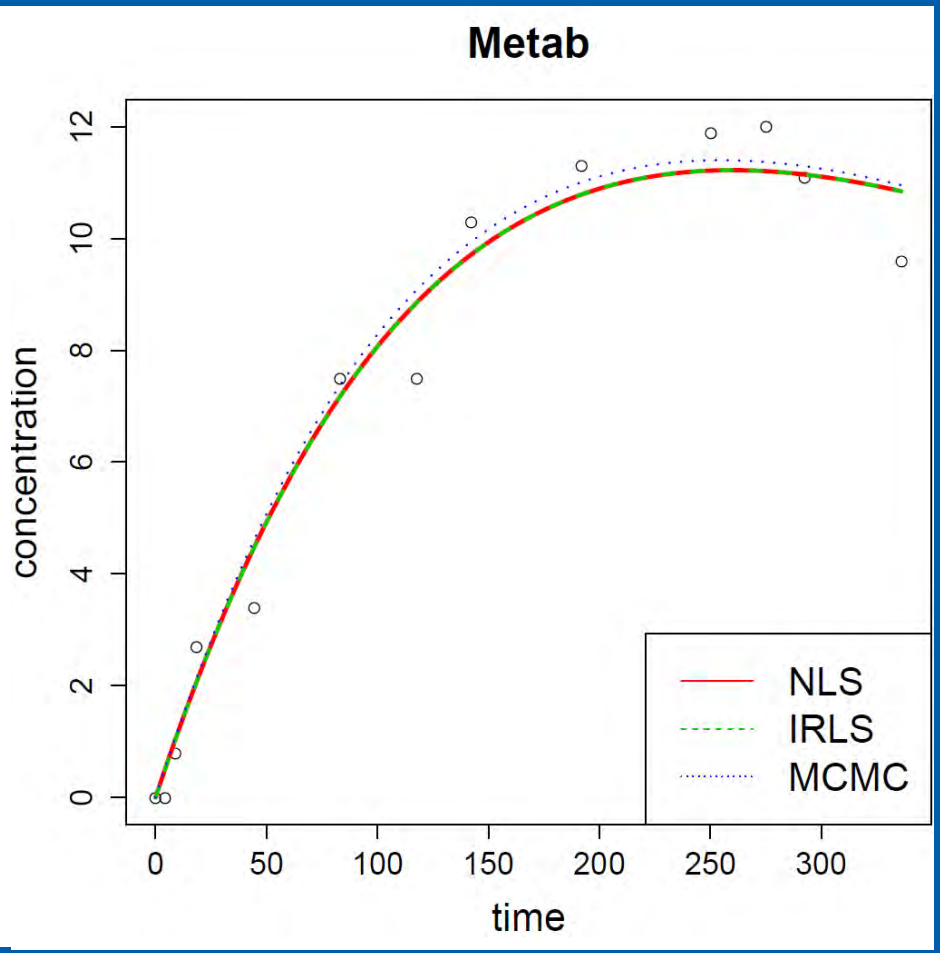

Parent
concentration
time
NLS
IRLS
MCMC
Metab
concentration
time
NLS
IRLS
MCMC

Example 1

Parameter	Algorithm	Estimate	Lower CI	Upper CI
k_p	NLS	0.0058	0.0047	0.0069
	IRLS	0.0058	0.0042	0.0074
	MCMC	0.0058	0.0043	0.0077
k_m	NLS	0.0084	-0.0178	0.0346
	IRLS	0.0084	0.0053	0.0115
	MCMC	0.0085	0.0058	0.0124
C	NLS	0.221	-0.278	0.719
	IRLS	0.219	0.130	0.309
	MCMC	0.223	0.150	0.352
M_0	NLS	194.6	179.9	209.4
	IRLS	194.8	174.0	215.6
	MCMC	194.3	172.7	216.2

Example 2

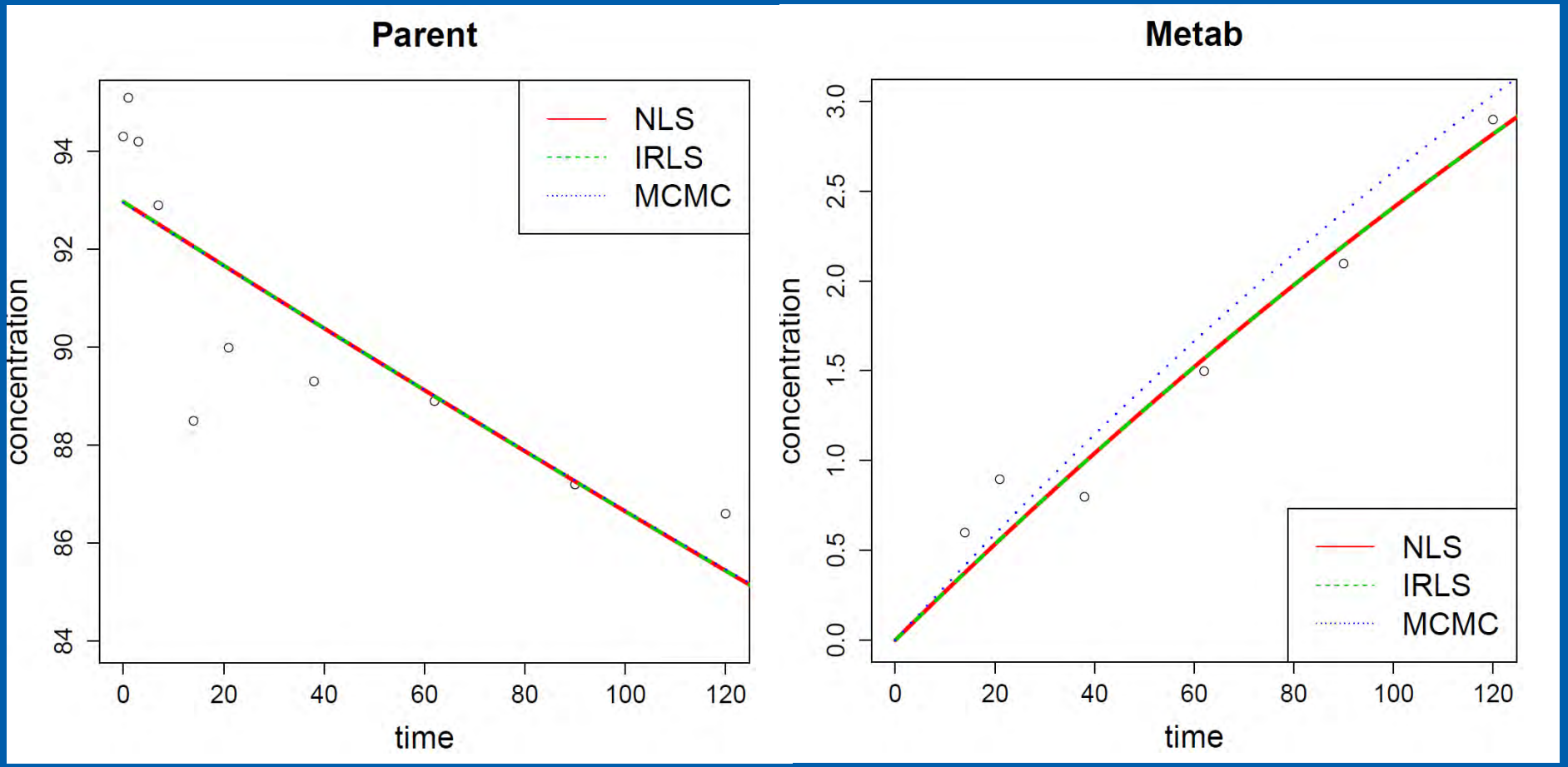

Parent
concentration
time
NLS
IRLS
MCMC
0
50
100
150
200
250
300


Metab
concentration
time
NLS
IRLS
MCMC
0
2
4
6
8
10
12
50
100
150
200
250
300

Example 2

Parameter	Algorithm	Estimate	Lower CI	Upper CI
k_p	NLS	0.0056	0.0046	0.0069
	IRLS	0.0055	0.0042	0.0074
	MCMC	0.0055	0.0043	0.0077
k_m	NLS	0.0025	-0.0172	0.0346
	IRLS	0.0026	0.0012	0.0115
	MCMC	0.0026	0.0013	0.0124
C	NLS	0.098	-0.167	0.719
	IRLS	0.100	0.068	0.309
	MCMC	0.101	0.074	0.352
M_0	NLS	219.8	204.9	234.6
	IRLS	219.1	198.1	240.1
	MCMC	218.7	197.5	240.8

Example 3


Parent
concentration
time
NLS
IRLS
MCMC
Metab
concentration
time
NLS
IRLS
MCMC

Example 3

Parameter	Algorithm	Estimate	Lower CI	Upper CI
k_p	NLS	0.0007	0.0005	0.0010
	IRLS	0.0007	0.0004	0.0011
	MCMC	0.0007	0.0003	0.0011
k_m	NLS	0.0019	-0.0470	0.0507
	IRLS	0.0019	-0.0044	0.0081
	MCMC	0.0022	-0.0050	0.0113
C	NLS	0.4183	-0.5871	1.424
	IRLS	0.4185	0.1698	0.667
	MCMC	0.4268	0.2412	1.011
M_0	NLS	92.97	204.9	94.14
	IRLS	92.96	198.1	94.63
	MCMC	92.96	197.5	94.68

References

- L. Görlitz, Z. Gao, W. Schmitt. 2011. Statistical Analysis of Chemical Transformation Kinetics using Markov-Chain Monte-Carlo Methods. Environ. Sci. Technol. 45:4429–4437

- Z. Gao, J.W. Green, J. Vanderborght, W. Schmitt. 2011. Improving Uncertainty Analysis in Kinetic Evaluations Using Iteratively Reweighted Least Squares. Environ. Toxicol. Chem. http://onlinelibrary.wiley.com/doi/10.1002/etc.630/abstract

Implementation

- The IRLS and MCMC procedures have been implemented in KinGUI2 and CAKE.
  - Both programs released in August 2011
  - Available free of charge

- Other software packages also contain these procedures.

Conclusions

- Confidence intervals estimated using the NLS approach can be overly wide when the error variance for parent is greater than for the metabolite.

- The IRLS and MCMC approaches provide more realistic estimates of confidence intervals when the error variance for parent is greater than for the metabolite.