Electronic Supplementary Material (ESI) for Journal of Analytical Atomic Spectrometry

Calibration and reverse calibration : R functions and examples

Aurélien THOMEN, Laurent REMUSAT, François ROBERT

06/18/2013

This supplementary information file provides recipe to perform an univariate calibration with a linear model, to calculate the prediction (and confidence) interval of the dependent variables, to reverse the calibration and the interval for a new measurement, to calculate the critical limit and its associated value in x axis. The code is written in R programming language [1]. R is free and open-source, dedicated to data mining and statistics and multi-platform (Windows, Mac OS X, Linux). It was found to be the most suitable way to share the calibration methods used in this paper. The data are shown in the Table 1.

1 R function

This section provides the functions to perform the calculation. The linear regression, prediction and confidence interval and linear model and interval inversion formula can be found in the book of [2, 3] the critical limit calculation can be found in [3].

```
# return a data.frame of the linear model of calibration and prediction interval
#       for content sequence
        # x             :       values of the standards
        # y             :       values of the measurements of the standards
        # content       :       unique values of the standards,
        #                       by default content = seq(range(x),len=length(x))
        # alpha         :       interval with a (1-alpha)100 level;
        #                       by default alpha = 0.05 for 95% confidance level
        # m             :       m = 1 by default to produce prediction interval
        #                       with a (1-alpha)100 level
        #                       m = Inf to produce confidence interval

interv  <- function( x, y,alpha, content=seq(min(x),max(x),len=2*length(unique(x))), m=1){
                        #linear model
                        model   <-      lm(y~x)$coef[1] + lm(y~x)$coef[2]*content
                        # variance of the residuals
                        res             <-      lm(y~x)$res
                        res.var <-      sum((res-mean(res))^2)/(length(x)-2)
                        pred    <-  qt(1-alpha/2, length(x)-2) * res.var^0.5*
                                (1/m+ 1/length(x) + (content-mean(x)) ^2/sum( (x-mean(x)) ^2 ) )^0.5
                        data.frame(     list(
                                        content = content, y.model = model,
                                        pred.up = model + pred, pred.low = model - pred
                                )       )
                        }
```

```
###############
# for reverse regression
# return the reverse value of a new mesure new.mes with its uncertainties
# return a warning message if the calibration is useless
#       g.limit > 0.2 by default (broad prediction interval)
rev.calib       <- function(new.mes,alpha, x, y, m=1, g.limit=0.2){
                #linear model
                lm.out  <-      lm( y ~ x )
                summary.lm.out  <-      summary(lm.out)
                # slope
                b1      <-      summary.lm.out$coef[2]
                #y-intercept
                b0      <-      summary.lm.out$coef[1]
                student.coef    <-      qt(1-alpha/2, length(x)-2)
                sxx     <-      sum ( (x- mean(x))^2 )
                res.var <-      summary.lm.out$sigma^2
                g       <-      (student.coef*res.var^0.5/b1)^2/sxx
                # diagnostic if calibration is useless print the the upper limit for g = 0.02
                if (g>0.2)
                        cat(paste("g>", g,"\n"))
                xchap   <-      (new.mes-b0)/b1
                num     <-      student.coef*res.var^.5/b1 *
                        ((xchap-mean(x))^2/sxx + (1/m+1/length(x))*(1-g))^0.5
                xu      <-      mean(x) +  ( xchap-mean(x) + num)/(1-g)
                xlow    <-      mean(x) +  ( xchap-mean(x) - num)/(1-g)

                data.frame(list(content = xchap , xu = xu, xlow = xlow))
                }

###############
# detection limit : critical limit lc
# return the critical limit lc and reverse regression to
#       return its corresponding value in x axis.
limit   <-      function(x, y, alpha){
                #linear model
                lm.out  <-      lm( y ~ x )
                summary.lm.out  <-      summary(lm.out)
                # slope
                b1      <-      summary.lm.out$coef[2]
                #y-intercept
                b0      <-      summary.lm.out$coef[1]
                student.coef    <-      qt(1-alpha/2, length(x)-2)
                sxx     <-      sum ( (x- mean(x))^2 )
                res.var <-      summary.lm.out$sigma^2
                # critical limit
                lc      <-      interv(x, y, 0.05, 0, 1)$pred.up
                # xc
                xc      <-      rev.calib(lc, alpha, x, y, m=1)$content
                data.frame(
                                list(xc=xc, lc = lc)
                                )
                }
```

2 Reverse calibration script for an R session

This section gives an example of reverse calibration to find the [N] content or the N/C ratio. The figure 1 shows the calibration of [N] mg.g^{-1} with CN^-/C^- and N/C with CN^-/C_2^-. The linear model, prediction interval and reversion of a new measurement, arbitrarily chosen for each calibration, is also displayed.

```
# the data file with a .txt extension is stored in a directory "yourdirectory" in
```

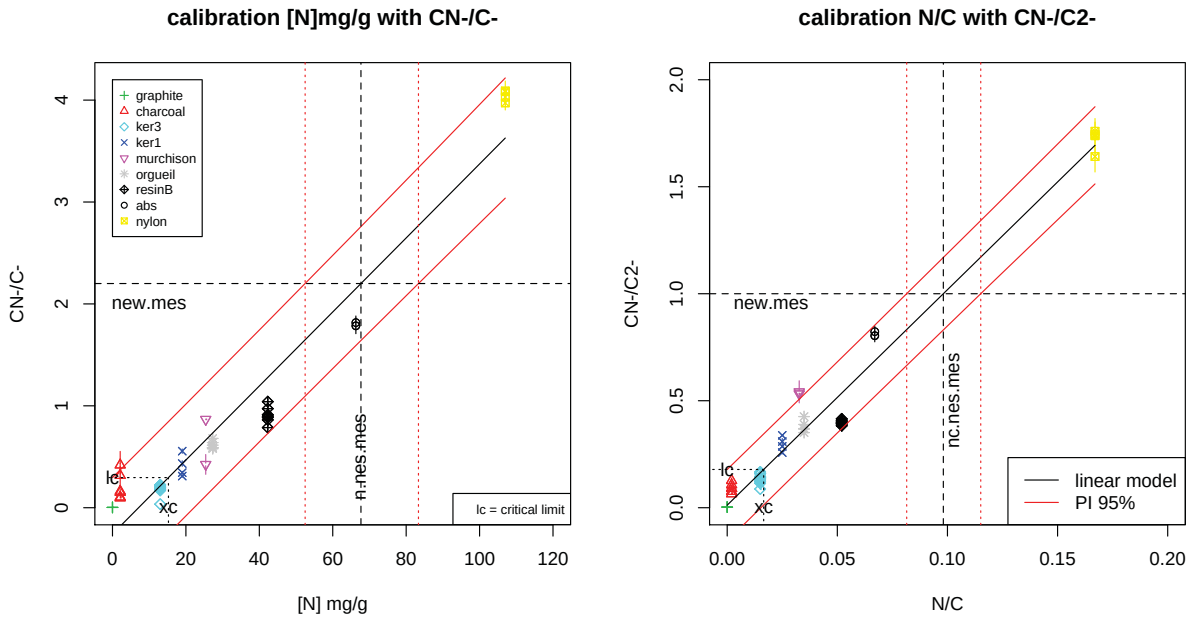

Figure 1: Calibration plot of the $[N]/mg.g^{-1}$ with CN^-/C^- and N/C atomic ratio with CN^-/C_2^-. The new.mes is inverted in **n.new.mes** and **nc.new.mes** with their uncertainties. **lc** is the critical limit and **xc** its reverse value.

```
#       your Desktop for instance (for Unix-like system) :
setwd("~/Desktop/yourdirectory")

# load package
library("gplots")

# load data from the data file "nitrogen_calib.txt". The data shown at the table 1 should be store inside th
d       <-      read.table("nitrogen_calib.txt")

# plot calibration data

layout(matrix(1:2,1,2))

plotCI( d$n_mgg, d$meancnc, uiw = 2*d$sd_cnc, sfrac=0, gap = 0.1,
                col = d$type, cex = .9, pch = as.numeric(d$type),
                xlim = c(0,120), ylim = c(0,4.2), xlab = "[N] mg/g", ylab = "CN-/C-",
                main="calibration [N]mg/g with CN-/C-")

legend(0,4.2, unique(d$type), col = unique(d$type), pch = unique(as.numeric(d$type)), cex=.7 )


# prediction band CN/C- vs. [N] mg/g
calib.model.cnc <- interv(d$n_mgg, d$meancnc, 0.05)
matlines(calib.model.cnc[,1], calib.model.cnc[,-1], , col=c(1, 2,2), lty=1)

##############################################
# reverse calibration for new.mes.cnc of CN-/C- mesured in a sample
```

```
new.mes.cnc <- 2.2

# on the plot CN-/C- vs. [N] mg/g
abline( h = new.mes.cnc, lty = 2)
text(10,new.mes.cnc-.2, "new.mes")
# reverse calibration and predition interval to obtain the N/C and
#       its uncertainties corresponding to new.mes.cnc2
# function rev.calib() do the work
n.nes.mes <- rev.calib(new.mes.cnc, 0.05, d$n_mgg, d$meancnc, 1)

abline( v = n.nes.mes, col=c(1,2,2), lty = c(2,3,3) )
text(n.nes.mes$content, 0.5, "n.nes.mes", srt=90)
##########################################
# detection limit for CN-/C-

l.cnc <- limit(d$n_mgg, d$meancnc, 0.05)

segments(l.cnc$xc,      -1, l.cnc$xc, l.cnc$lc, lty=3)
text( l.cnc$xc, 0, "xc")

segments(-1, l.cnc$lc, l.cnc$xc, l.cnc$lc, lty=3)
text( 0, l.cnc$lc, "lc")

legend( "bottomright", "lc = critical limit", cex=.7 )


#############

plotCI( d$nc, d$meancnc2, uiw = 2*d$sd_cnc2, sfrac=0, gap = 0.1,
                col = d$type, cex = .9, pch = as.numeric(d$type),
                xlim = c(0,.2), ylim = c(0,2), xlab = "N/C", ylab = "CN-/C2-",
                main="calibration [N]mg/g with CN-/C2-")

# prediction band CN-/C2- vs N/C atomic ratio
calib.model.cnc2        <-      interv(d$nc,    d$meancnc2, 0.05)
matlines(calib.model.cnc2[,1], calib.model.cnc2[,-1], col=c(1, 2,2), lty=1)

# legend for the calibration model and prediction interval PI
legend("bottomright",c("linear model", "PI 95%"), col=c(1, 2), lty=1)

##########################################
# reverse calibration for new.mes.cnc2 of CN-/C2- measured in a sample
new.mes.cnc2    <-      1

# on the plot CN-/C2- vs. N/C
abline( h = new.mes.cnc2, lty = 2)

# reverse calibration and prediction interval to obtain the N/C and
#       its uncertainties corresponding to new.mes.cnc2
# function rev.calib() do the work
nc.nes.mes      <-      rev.calib(new.mes.cnc2, 0.05, d$nc, d$meancnc2, 1)

abline( v = nc.nes.mes, col=c(1,2,2), , lty = c(2,3,3) )

##########################################
# detection limit for CN-/C2-

l.cnc2 <- limit(d$nc, d$meancnc2, 0.05)

segments(l.cnc2$xc,     -1, l.cnc2$xc, l.cnc2$lc, lty=3)
text( l.cnc2$xc, 0, "xc")

segments(-1, l.cnc2$lc, l.cnc2$xc,      l.cnc2$lc, lty=3)
text( 0, l.cnc2$lc, "lc")
```

References

[1] R Core Team R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013, http://www.R-project.org

[2] Draper,N.R., Smith, H. Applied regression analysis, Third edition, Wiley Series in probability and statistics, 1998

[3] Lavagnini, I., Magno, F. A statistical overview on univariate calibration, inverse regression, and detection limits : application to gas chromatography/mass spectrometry technique, Mass Spectrometry Review, 26, 1-18, 2007

session	type	N/C	[N]/mgg	$^{12}C^{14}N^-/^{12}C^-$	$^{12}C^{14}N^-/^{12}C^-$
january	graphite	0.000	0.000	0.003 ± 0.000	0.003 ± 0.000
january	graphite	0.000	0.000	0.003 ± 0.000	0.003 ± 0.000
july	charcoal	0.002	2.100	0.104 ± 0.002	0.065 ± 0.001
july	charcoal	0.002	2.100	0.162 ± 0.010	0.090 ± 0.003
july	charcoal	0.002	2.100	0.150 ± 0.008	0.090 ± 0.003
july	charcoal	0.002	2.100	0.096 ± 0.006	0.076 ± 0.003
july	charcoal	0.002	2.100	0.419 ± 0.067	0.127 ± 0.006
july	charcoal	0.002	2.100	0.316 ± 0.060	0.107 ± 0.008
july	type 3 kerogen	0.015	13.000	0.194 ± 0.000	0.162 ± 0.001
july	type 3 kerogen	0.015	13.000	0.178 ± 0.001	0.160 ± 0.001
july	type 3 kerogen	0.015	13.000	0.222 ± 0.001	0.159 ± 0.001
july	type 3 kerogen	0.015	13.000	0.200 ± 0.008	0.167 ± 0.005
january	type 3 kerogen	0.015	13.000	0.212 ± 0.001	0.114 ± 0.000
january	type 3 kerogen	0.015	13.000	0.209 ± 0.001	0.143 ± 0.001
january	type 3 kerogen	0.015	13.000	0.192 ± 0.004	0.132 ± 0.001
january	type 3 kerogen	0.015	13.000	0.224 ± 0.001	0.135 ± 0.000
january	type 3 kerogen	0.015	13.000	0.169 ± 0.001	0.124 ± 0.001
january	type 3 kerogen	0.015	13.000	0.182 ± 0.004	0.121 ± 0.001
january	type 3 kerogen	0.015	13.000	0.187 ± 0.001	0.141 ± 0.000
january	type 3 kerogen	0.015	13.000	0.035 ± 0.002	0.087 ± 0.001
january	type 3 kerogen	0.015	13.000	0.223 ± 0.001	0.143 ± 0.000
january	type 3 kerogen	0.015	13.000	0.198 ± 0.000	0.146 ± 0.000
january	type 3 kerogen	0.015	13.000	0.180 ± 0.000	0.138 ± 0.002
july	type 1 kerogen	0.025	19.000	0.344 ± 0.006	0.287 ± 0.002
july	type 1 kerogen	0.025	19.000	0.555 ± 0.011	0.338 ± 0.001
july	type 1 kerogen	0.025	19.000	0.434 ± 0.013	0.309 ± 0.007
july	type 1 kerogen	0.025	19.000	0.310 ± 0.005	0.258 ± 0.003
july	Murchison IOM	0.033	25.400	0.425 ± 0.049	0.542 ± 0.026
january	Murchison IOM	0.033	25.400	0.867 ± 0.001	0.532 ± 0.001
january	Orgueil IOM	0.035	27.300	0.680 ± 0.001	0.368 ± 0.000
january	Orgueil IOM	0.035	27.300	0.590 ± 0.001	0.370 ± 0.001
january	Orgueil IOM	0.035	27.300	0.590 ± 0.001	0.351 ± 0.001
january	Orgueil IOM	0.035	27.300	0.579 ± 0.001	0.369 ± 0.000
january	Orgueil IOM	0.035	27.300	0.614 ± 0.001	0.389 ± 0.001
january	Orgueil IOM	0.035	27.300	0.639 ± 0.006	0.426 ± 0.003
january	Resin B	0.052	42.300	0.885 ± 0.008	0.383 ± 0.003
january	Resin B	0.052	42.300	0.862 ± 0.004	0.391 ± 0.004
january	Resin B	0.052	42.300	0.972 ± 0.009	0.401 ± 0.004
january	Resin B	0.052	42.300	0.899 ± 0.004	0.409 ± 0.006
january	Resin B	0.052	42.300	0.913 ± 0.014	0.415 ± 0.008
january	Resin B	0.052	42.300	1.041 ± 0.014	0.398 ± 0.003
january	Resin B	0.052	42.300	0.787 ± 0.009	0.393 ± 0.005
july	ABS	0.067	66.300	1.816 ± 0.032	0.823 ± 0.013
july	ABS	0.067	66.300	1.785 ± 0.038	0.804 ± 0.014
july	nylon	0.167	107.000	4.083 ± 0.017	1.738 ± 0.040
july	nylon	0.167	107.000	3.973 ± 0.034	1.641 ± 0.036
july	nylon	0.167	107.000	4.030 ± 0.023	1.759 ± 0.021
july	nylon	0.167	107.000	4.090 ± 0.048	1.744 ± 0.014

Table 1: $^{12}C^{14}N^-/^{12}C^-$ and $^{12}C^{14}N^-/^{12}C^-$ measurements of the standards. One row of the table is a single measurement or image.