The influence of the reference method on the results of the secondary method via calibration

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Direct methods

measure the property of the sample
or
the analyte as such.

Direct methods are therefore also called primary methods and often serve as reference methods.

Indirect methods

measure something that depends on the extent of the property or the concentration or amount of the analyte. They are therefore also called secondary methods.

Indirect methods need therefore relation to a direct method (to which they are “referred”)

in a way that

the value measured allows a conclusion to the property of the sample or the concentration or the amount of the analyte.

Samples with a known property (analysed with a “reference” method)
are measured with an indirect method and
the value found is related to the “true” value.

Such data pairs are plotted versus each other and a regression curve (mostly a straight line) is laid through these data points.

This is the calibration line.

When chemometric methods are applied, the analytical data points are calculated from many measurements each.
These values are often called "predicted values" and are plotted against the "true values" obtained by the reference method.

So, where is the problem ?

The whole approach depends on the “correctness” of the reference method,

because it is always possible to draw a regression line through data points.

It is therefore essential that the reference method be “correct”.

Example: Water determination

The Karl Fischer titration (KFT) detects water selectively,

whereas drying techniques determine mass loss under the applied conditions (other volatiles are also included and strongly bound water is not detected completely).

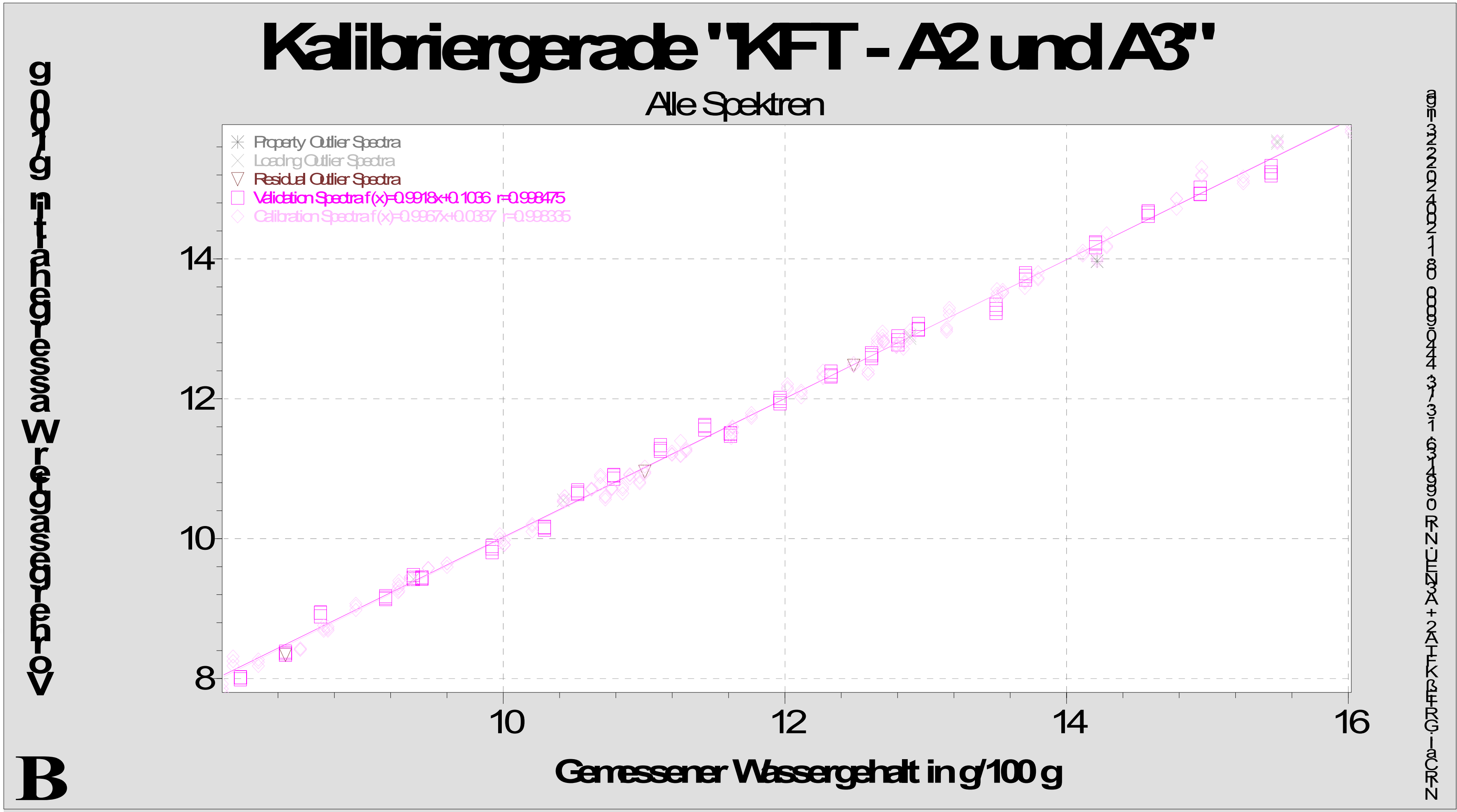
Near infrared (NIR) spectra depend on water content.

NIR calibrations should therefore be based on a reference method that really determines water and nothing but water (like the Karl Fischer titration).

Calibration can, however, also be carried out on the basis of a drying technique.

Chemometrics will always find a calibration line (even when random numbers are used !).

NIR calibration line for water content of wheat semolina, based on Karl Fischer titration

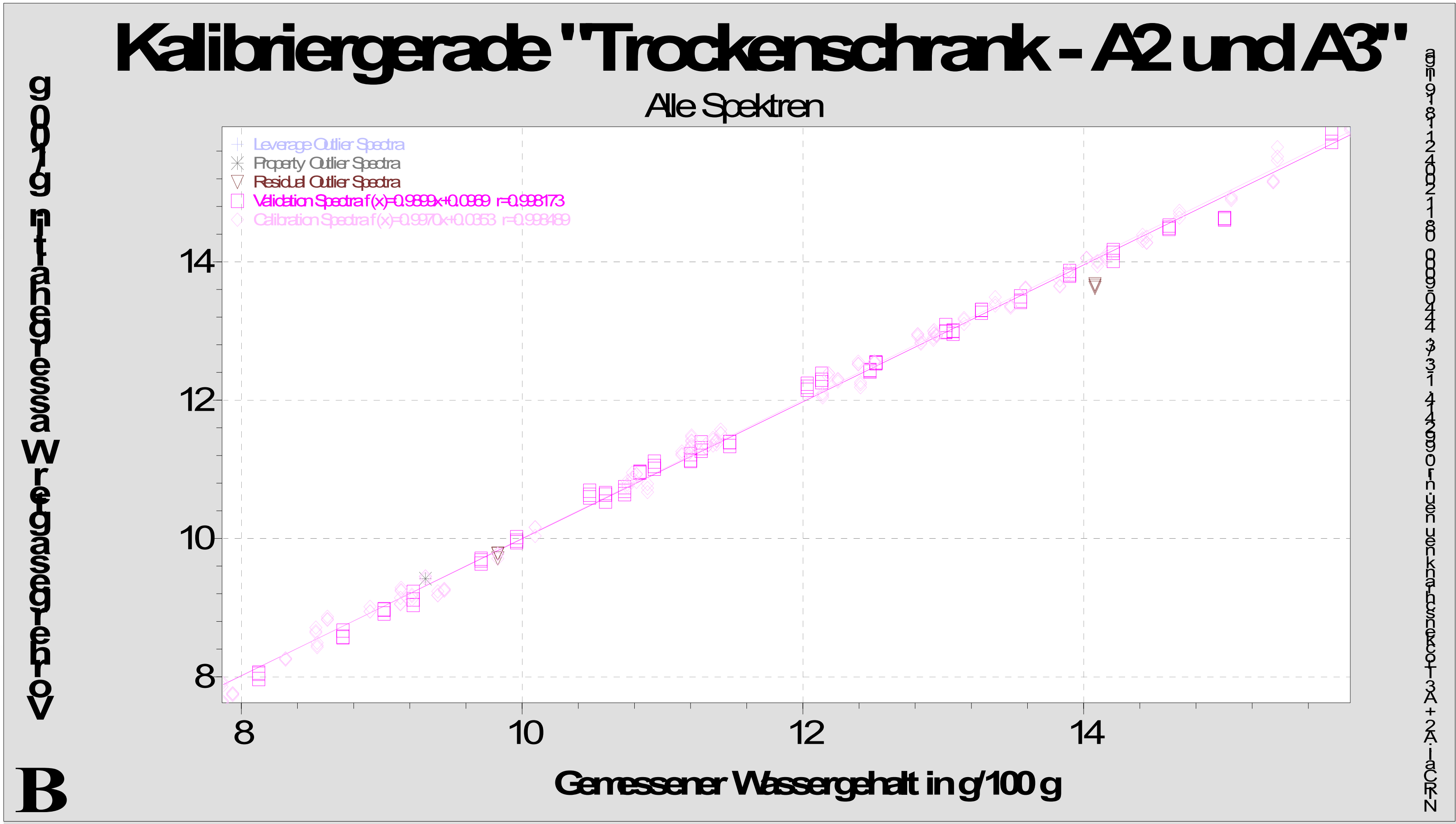


Water content in g/100 g predicted by NIR spectroscopy

Water content in g/100g measured by KFT (”true value“)

NIR calibration line for water content of wheat semolina, based on oven drying

Water content in g/100 g predicted by NIR spectroscopy



Water content in g/100g measured by oven drying (”true value“)

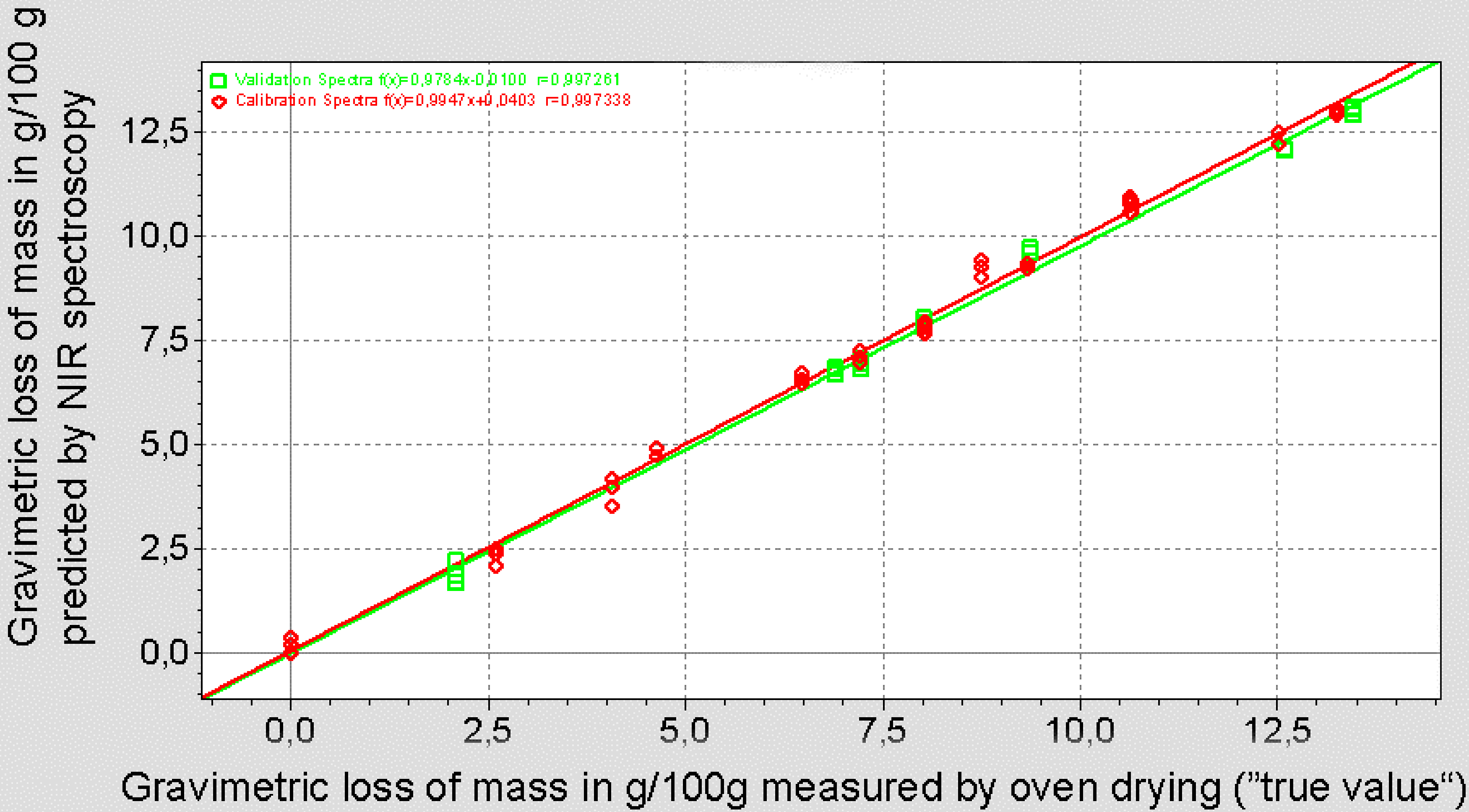
Curves are nearly identical.

Results follow the respective calibration curve (KFT slightly better than OD) and are as correct (or not) as the reference method.

In this case the differences are only small.

Another example:

Lactoserum
containing much lactose



Calibration against gravimetric mass lost by drying at 145 °C of Lactoserum (Euvoserum from Nestlé)

The calibration is acceptable if the mass loss on drying is really the property to be analysed.

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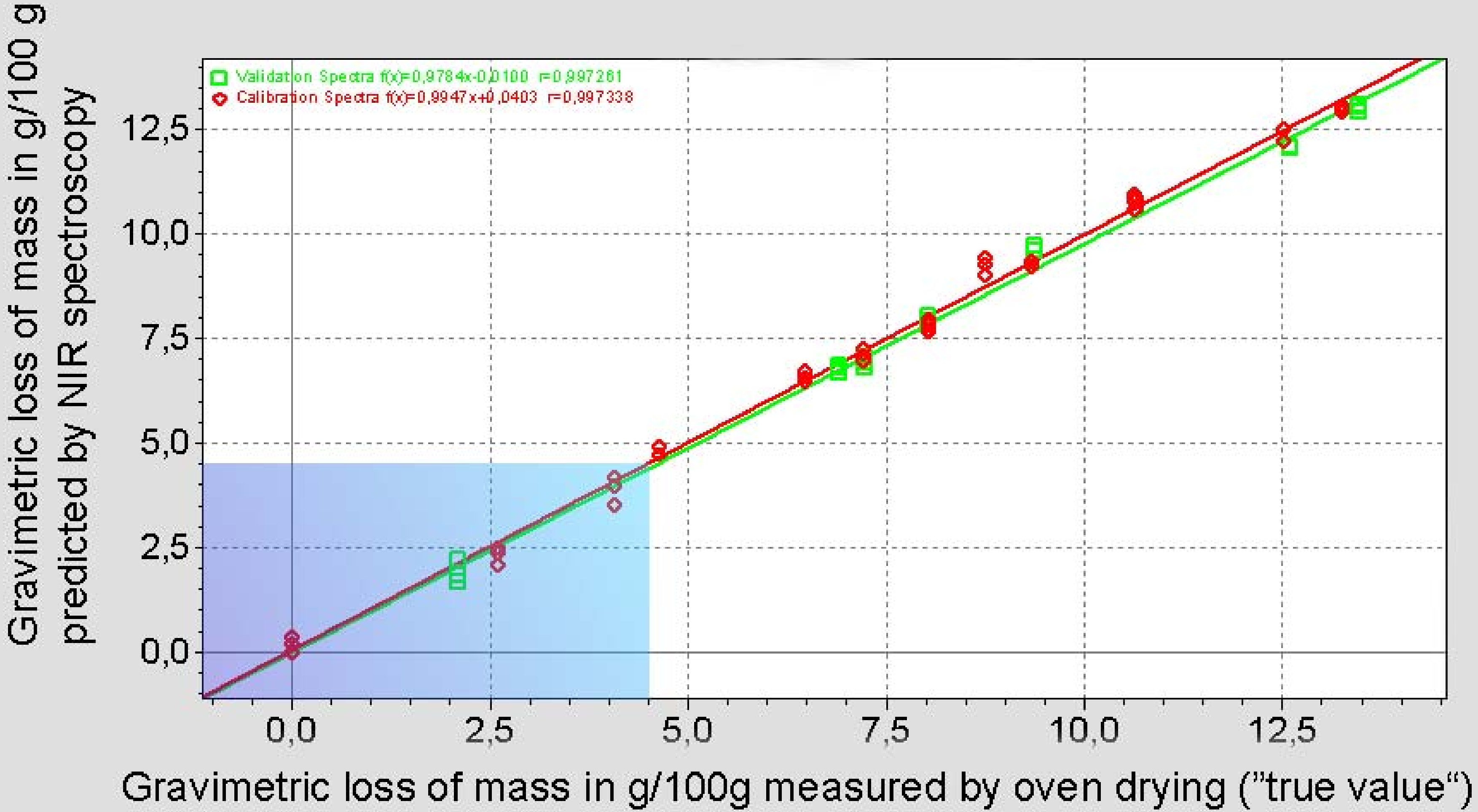
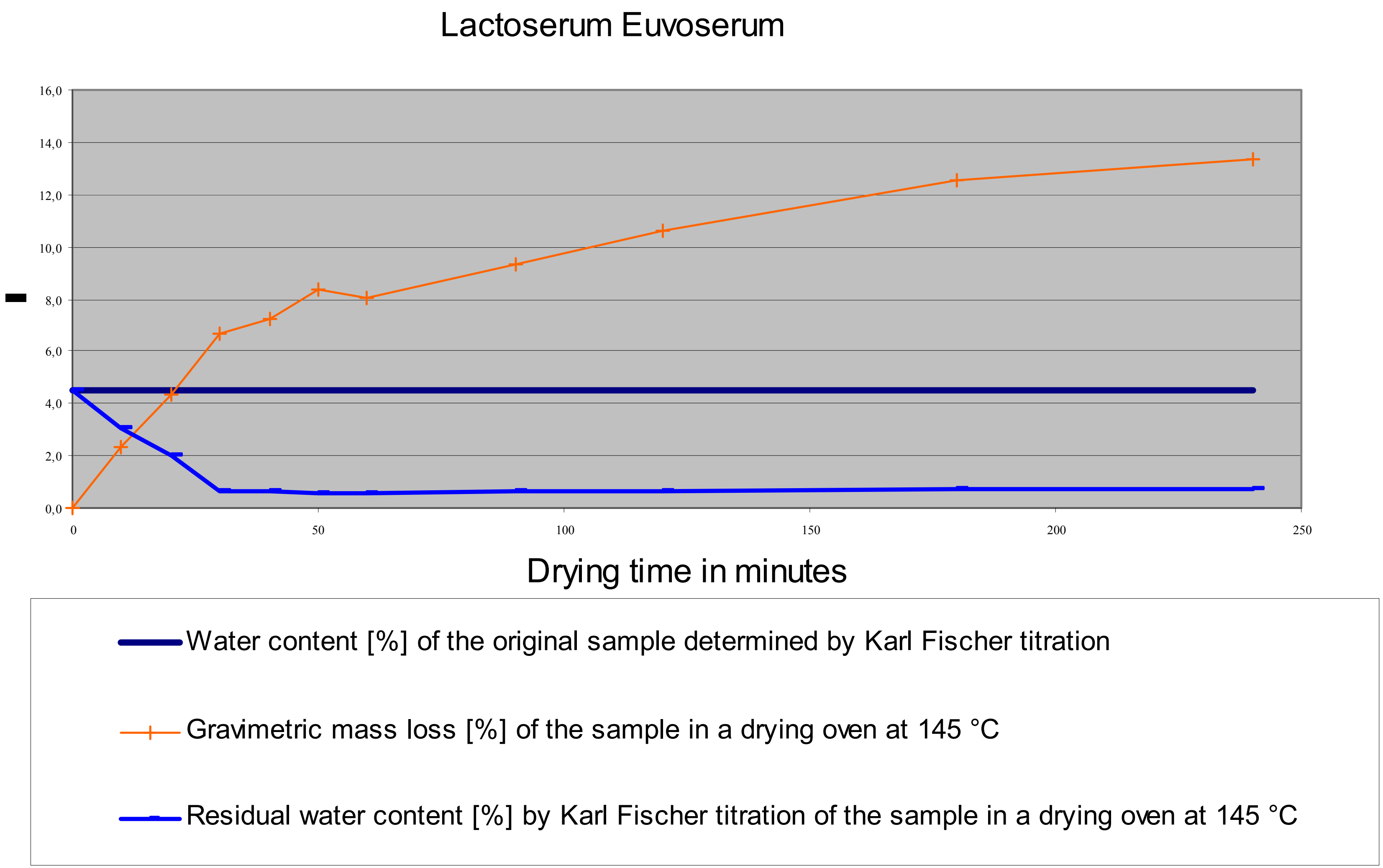
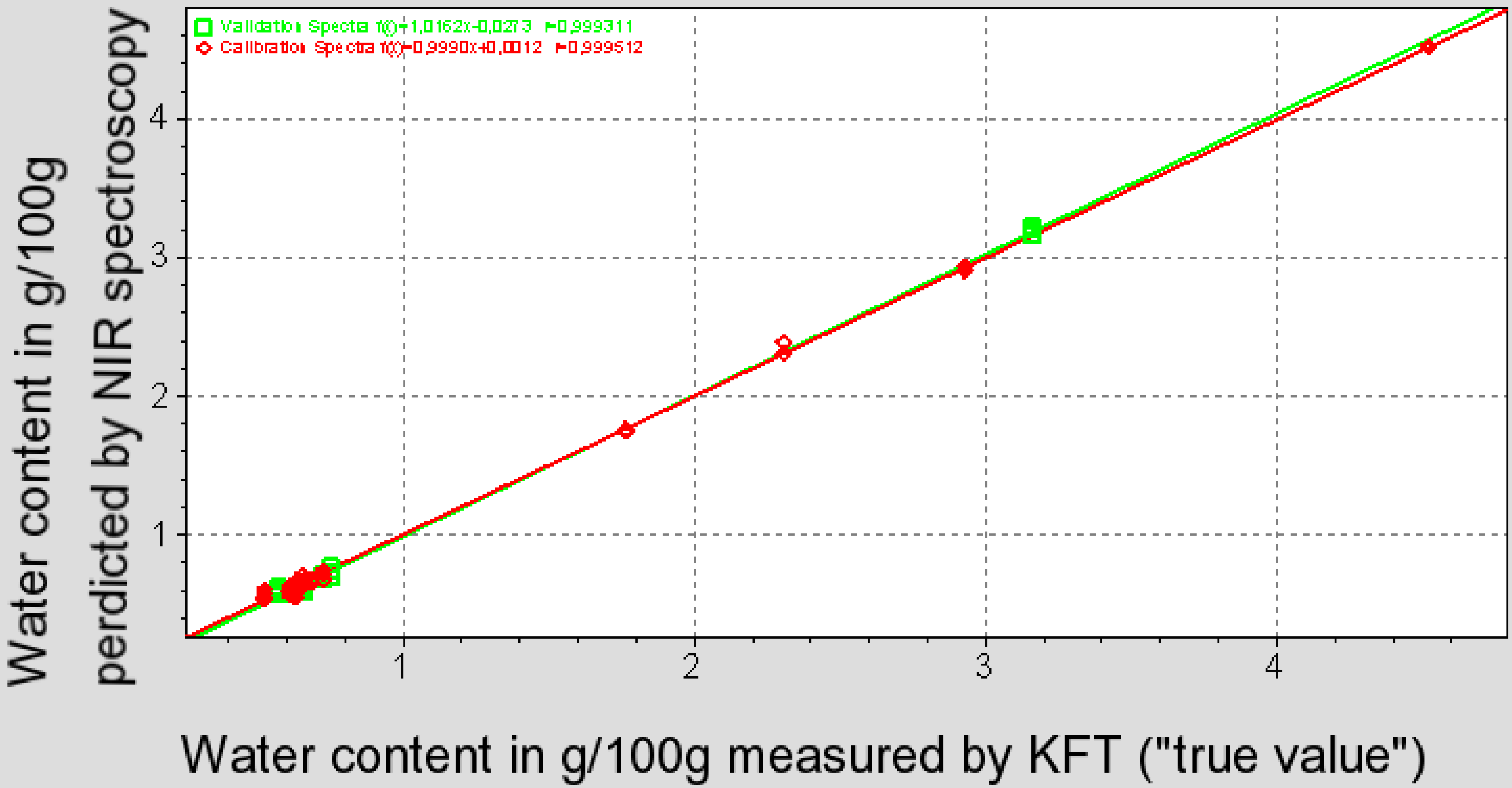
really the property to be analysed.

It is, however, useless
if water content is to be analysed.



Calibration against gravimetric mass lost by drying at 145 °C of Lactoserum (Euvoserum from Nestlé)


Lactoserum Euvoserum
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
0
50
100
150
200
250
Drying time in minutes
Water content [%] of the original sample determined by Karl Fischer titration
Gravimetric mass loss [%] of the sample in a drying oven at 145 °C
Residual water content [%] by Karl Fischer titration of the sample in a drying oven at 145 °C

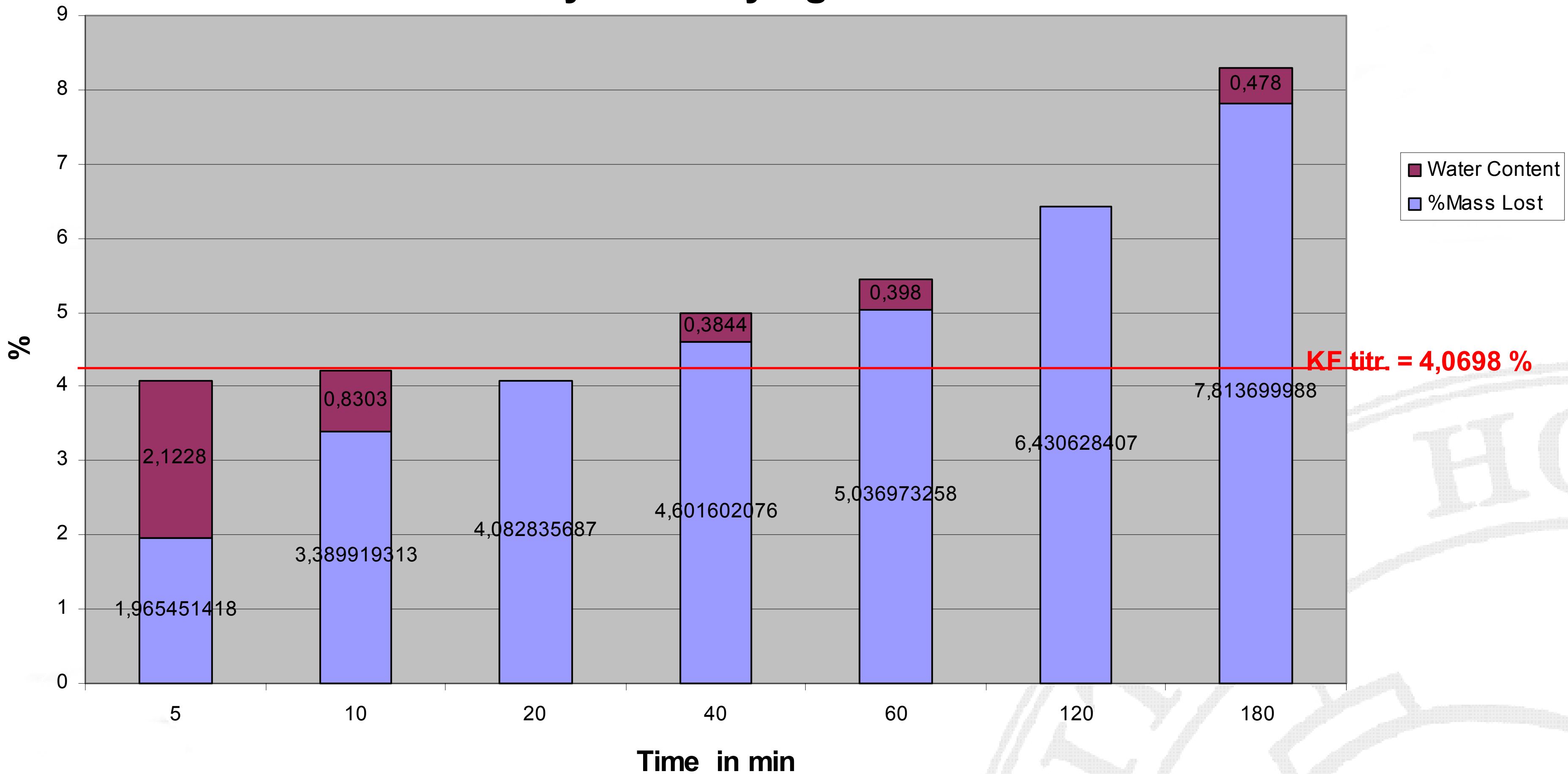


Calibration against water content determined by
Karl Fischer titration of Lactoserum (Euvoserum from Nestlé)

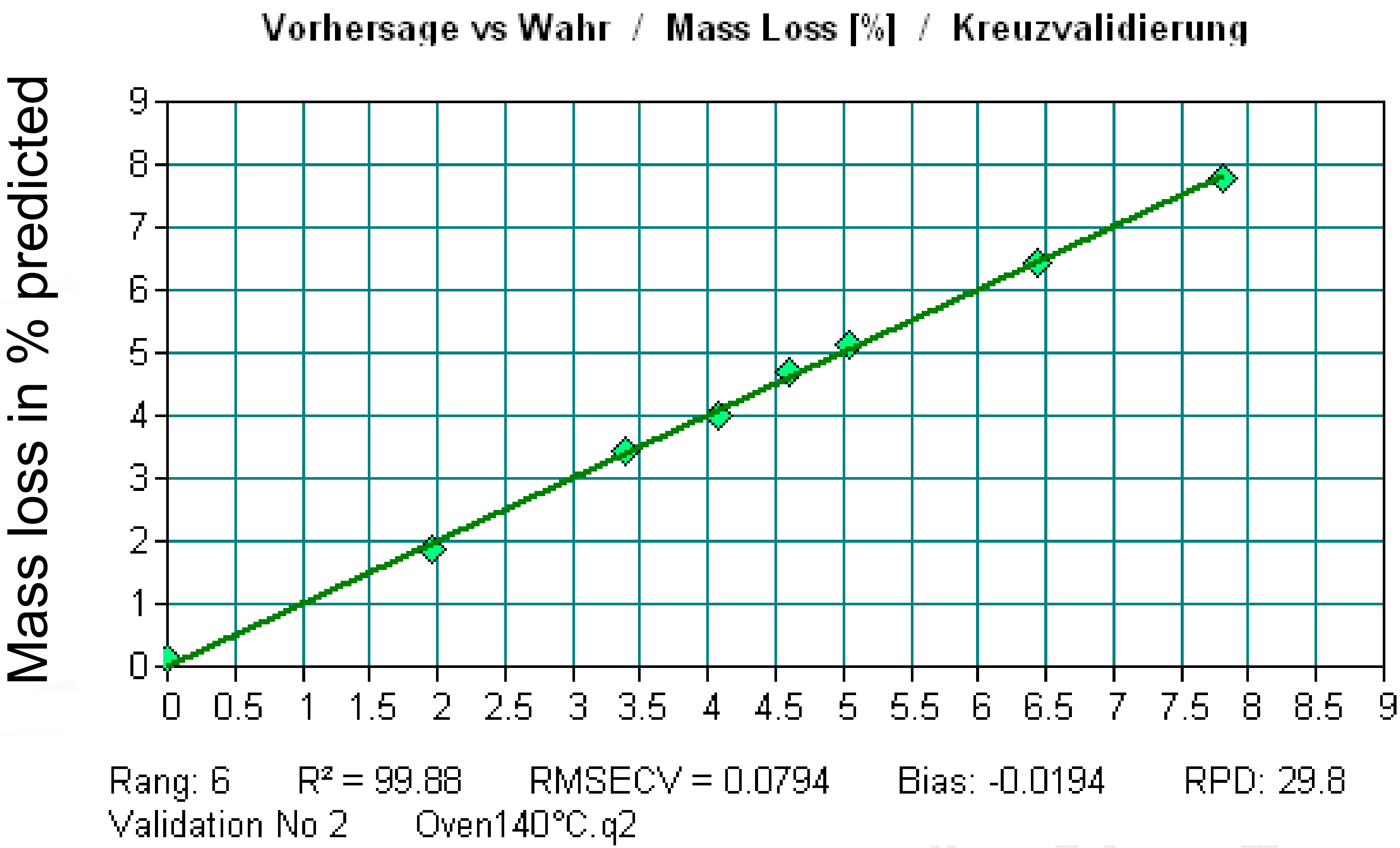
A further example:

Lasana, a baby food

Mass loss and water content in % of Lasana samples by oven drying at 140 °C

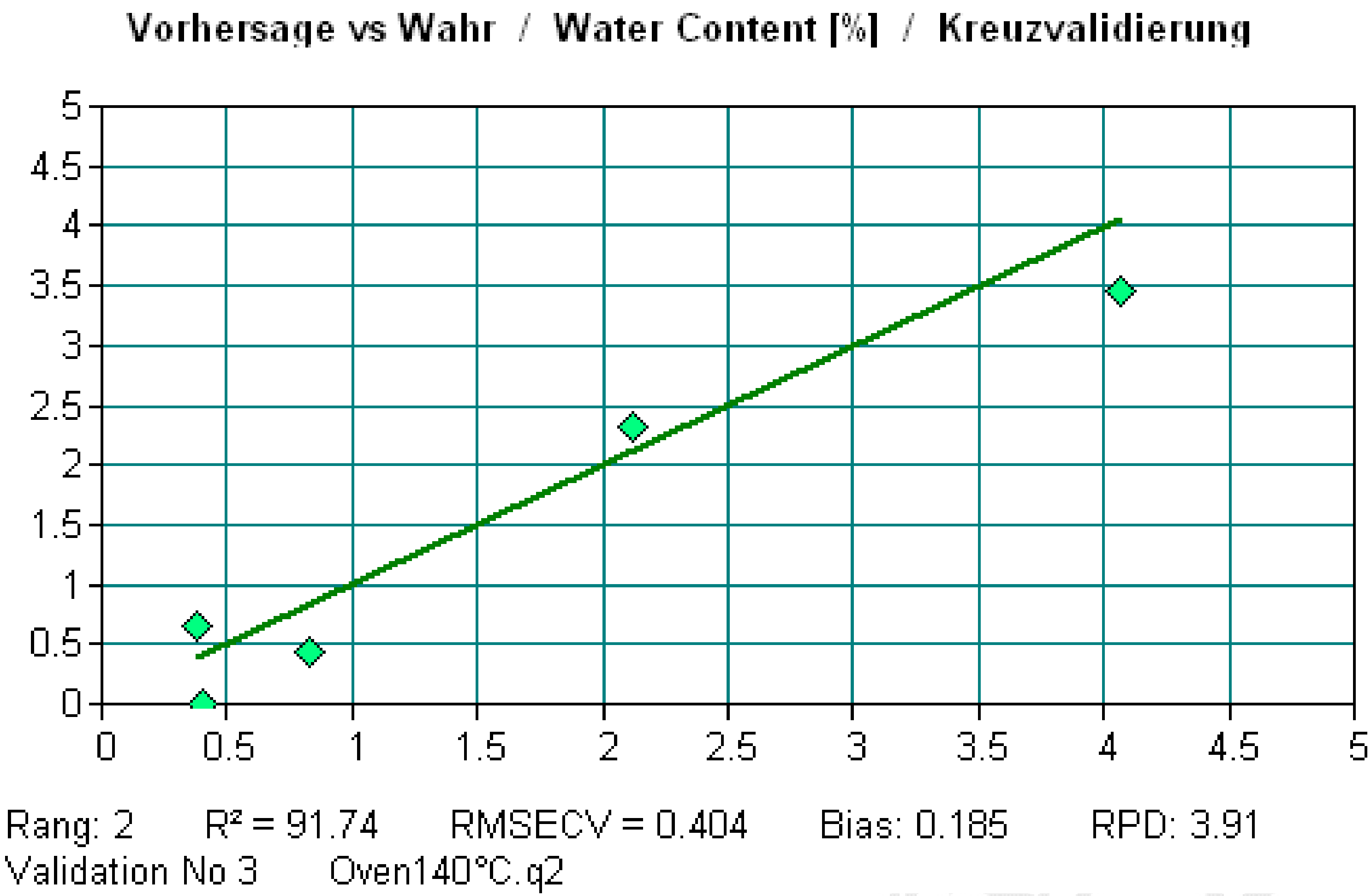


Calibration of mass loss against mass loss



Mass loss in % measured by oven drying at 140 °C

Calibration water content against water content (of dried product!)

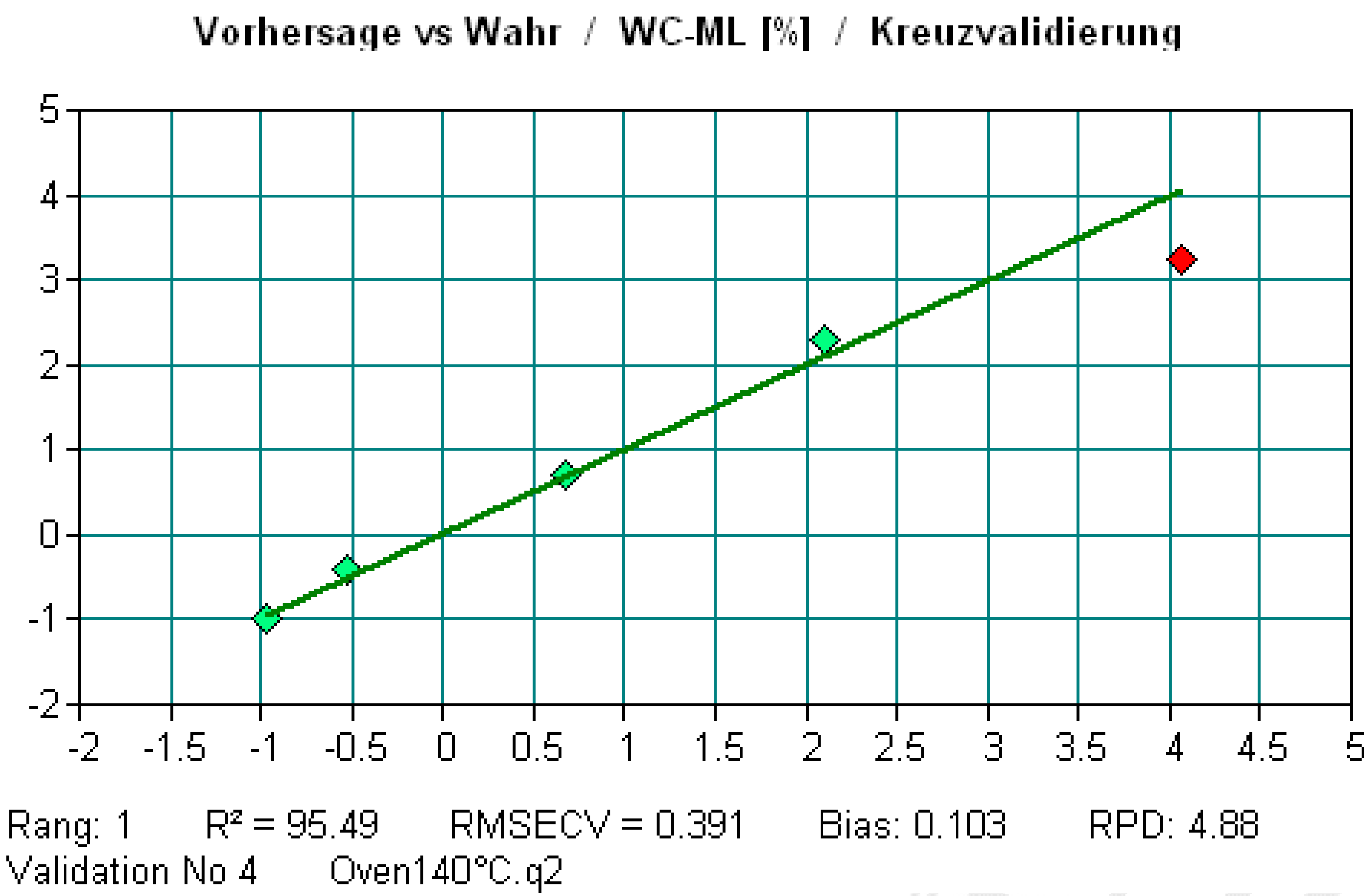


Water content in % predicted

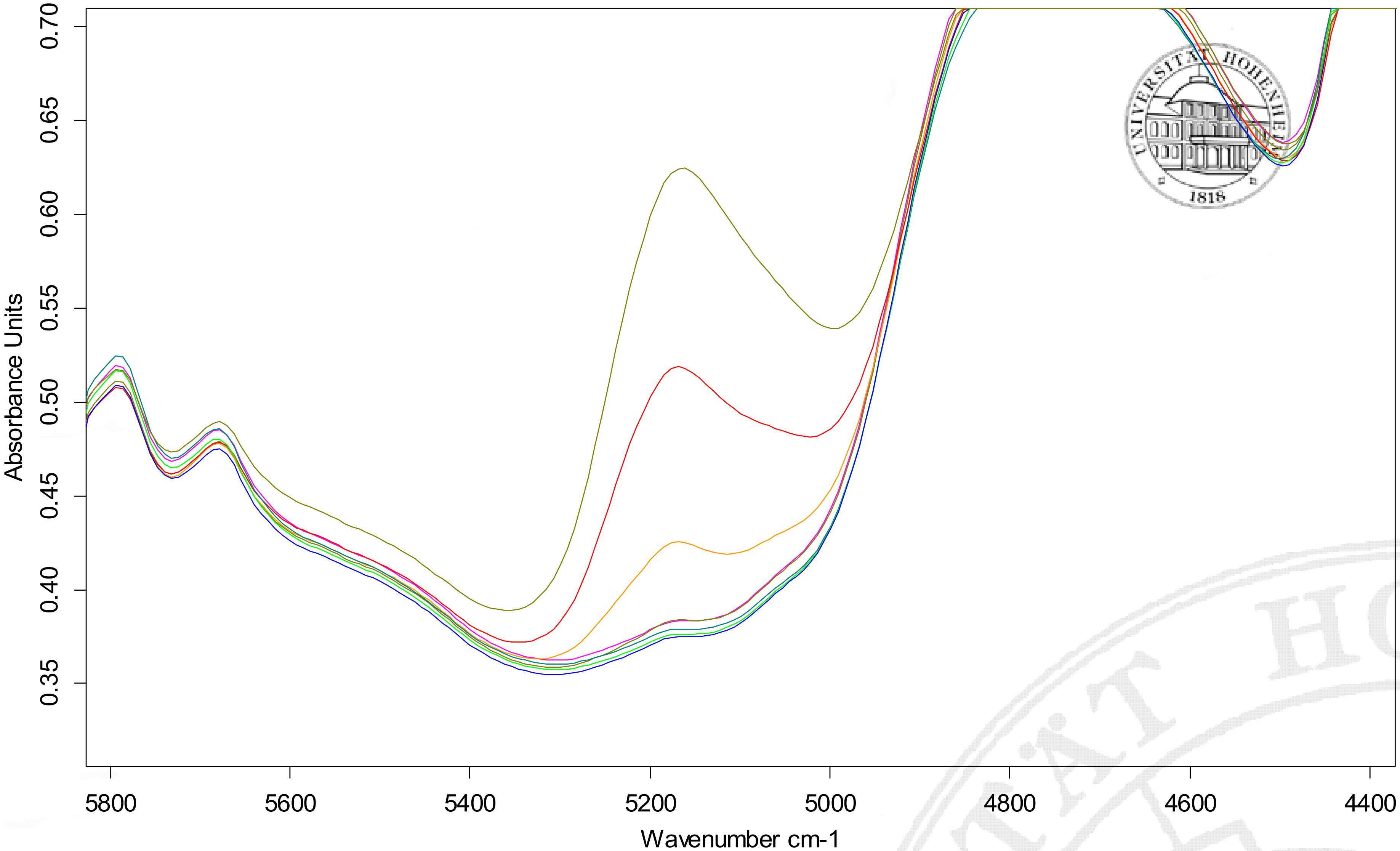
Water content in % measured after drying at 140 °C

Calibration true water content minus mass loss against true water content minus mass loss

True water content minus mass loss in % predicted

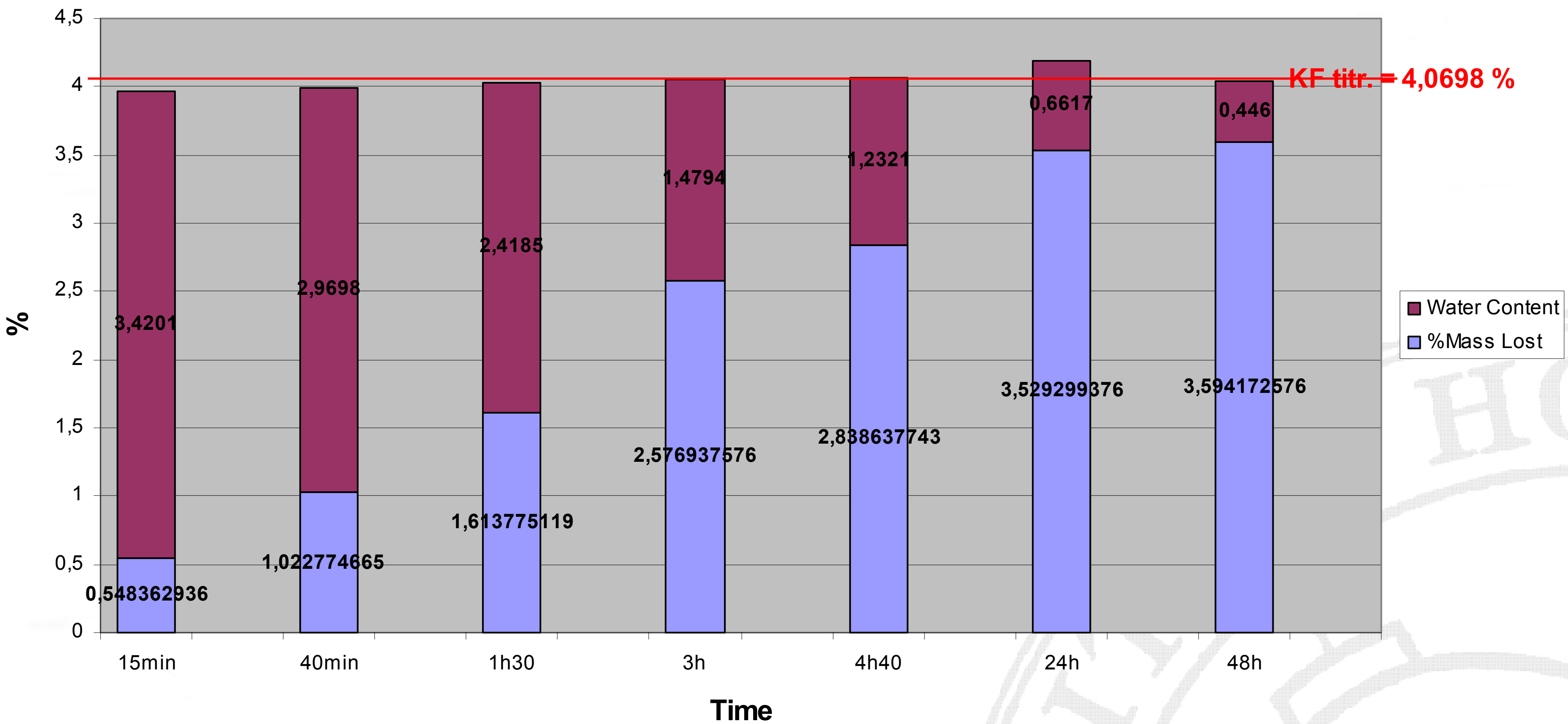


True water content minus mass loss in % measured



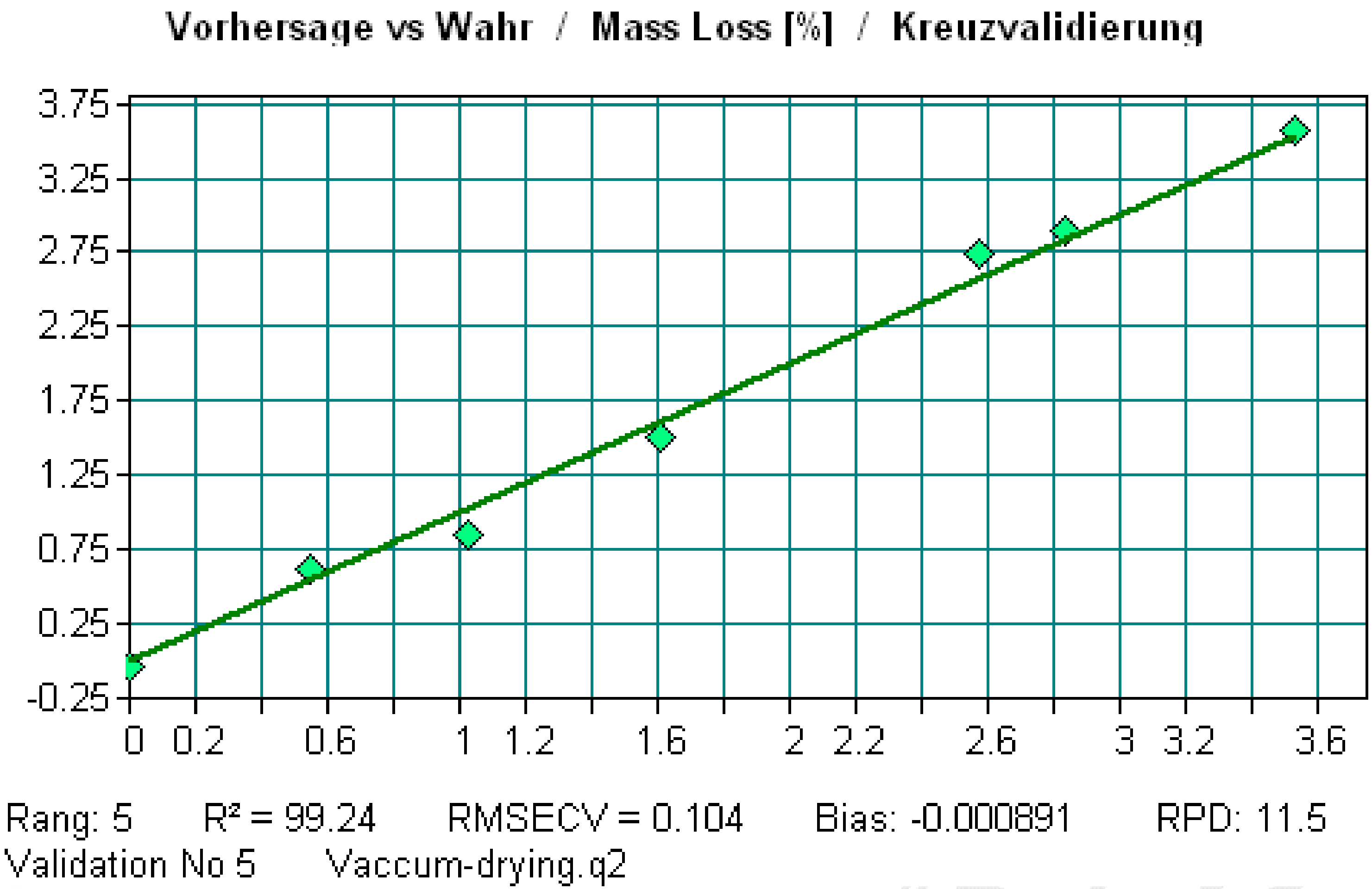
D:\Clement\Lasana original.0	Lasana original	sample form	08.02.2008
D:\Clement\Lasana oven drying 140° 1h.0	Lasana oven drying 140° 1h	sample form	18.02.2008
D:\Clement\Lasana oven drying 140° 2h.0	Lasana oven drying 140° 2h	sample form	15.02.2008
D:\Clement\Lasana oven drying 140° 3h.0	Lasana oven drying 140° 3h	sample form	18.02.2008
D:\Clement\Lasana oven drying 140° 5min.0	Lasana oven drying 140° 5min	sample form	15.02.2008
D:\Clement\Lasana oven drying 140° 10min.0	Lasana oven drying 140° 10min	sample form	15.02.2008
D:\Clement\Lasana oven drying 140° 20min.0	Lasana oven drying 140° 20min	sample form	15.02.2008
D:\Clement\Lasana oven drying 140° 40min.0	Lasana oven drying 140° 40min	sample form	15.02.2008

Mass loss and water content in % of Lasana samples by vacuum-oven drying at 48 °C with P_2O_5



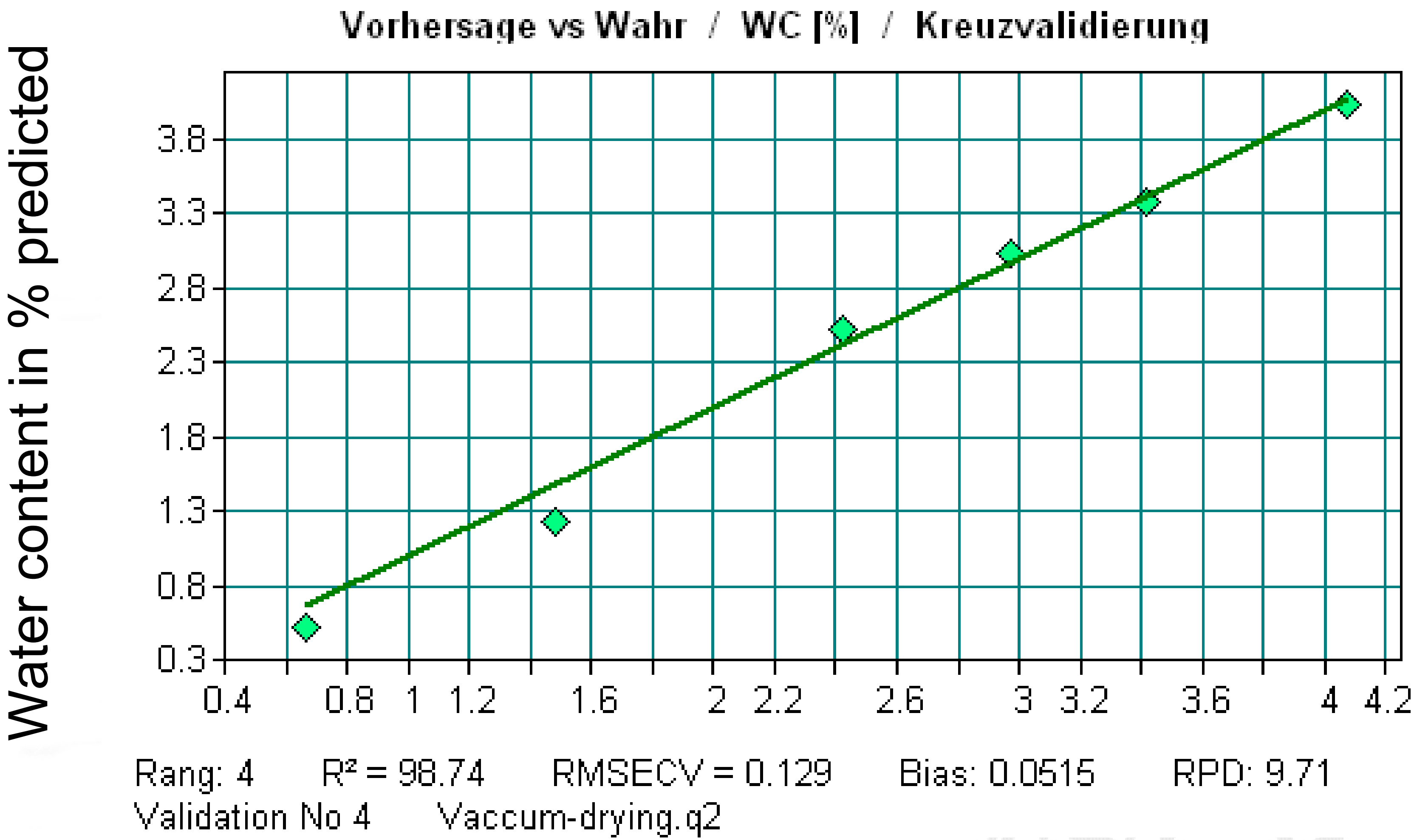
Calibration of mass loss against mass loss

Mass loss in % predicted



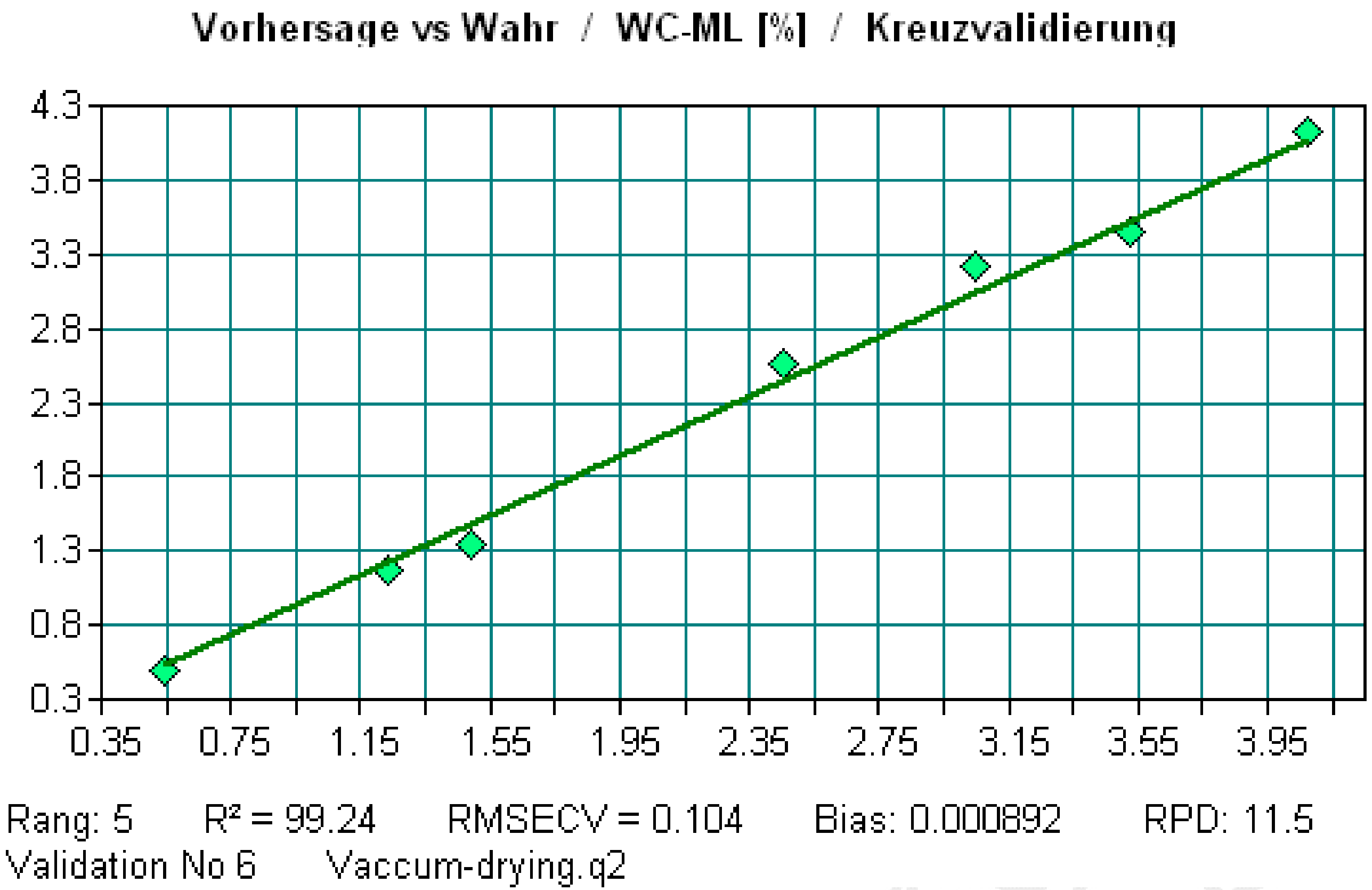
Mass loss in % measured by vacuum-oven drying at 48 °C using P_2O_5

Calibration water content against water content (of dried product!)



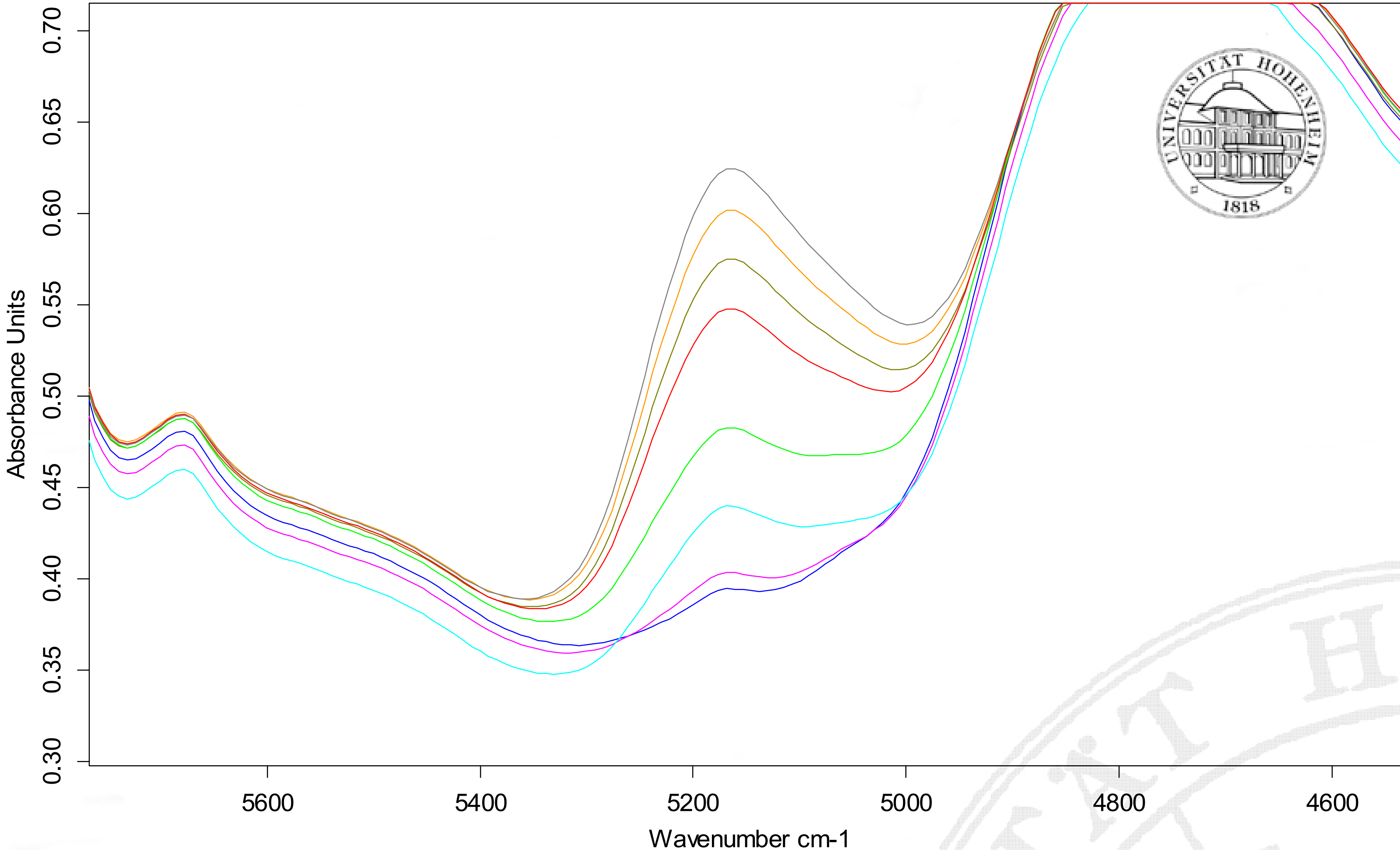
Water content in % measured after vacuum-oven drying at 48 °C using P_2O_5

Calibration true water content minus mass loss against true water content minus mass loss



True water content minus mass loss in % predicted

True water content minus mass loss in % measured



D:\Clement\Lasana original.0	Lasana original	sample form	08.02.2008
D:\Clement\Lasana vacuum Jo 1h30.0	Lasana vacuum Jo 1h30	sample form	11.03.2008
D:\Clement\Lasana vacuum Jo 3h.0	Lasana vacuum Jo 3h	sample form	11.03.2008
D:\Clement\Lasana vacuum Jo 4h40.0	Lasana vacuum Jo 4h40	sample form	11.03.2008
D:\Clement\Lasana vacuum Jo 15min.0	Lasana vacuum Jo 15min	sample form	11.03.2008
D:\Clement\Lasana vacuum Jo 24H.0	Lasana vacuum Jo 24H	sample form	12.03.2008
D:\Clement\Lasana vacuum Jo 40min.0	Lasana vacuum Jo 40min	sample form	11.03.2008
D:\Clement\Lasana vacuum Jo 48H.0	Lasana vacuum Jo 48H	sample form	13.03.2008

Summary:

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Be sure to have good references !