Increasing the Reliability of Untargeted Metabolomics

by Using Natural Stable ^{13}C Isotopes

Gunnar Libiseller[1], *Michaela Dvorzak*[2], *Ulrike Kleb*[2], *Alexander Fauland*[1], *Frank Sinner*[1,3], *Thomas Pieber*[1,3], *Christoph Magnes*[1*]

CONTACT

1
JOANNEUM RESEARCH
Forschungsgesellschaft mbH

HEALTH
Institute for Biomedicine
and Health Sciences

***Christoph Magnes**

Neue Stiftingtalstrasse 2
8010 Graz, Austria

Phone: +43 316 876-42 01
Fax: +43 316 876 9-42 01

christoph.magnes@joanneum.at

health@joanneum.at
www.joanneum.at/health

2
JOANNEUM RESEARCH
Forschungsgesellschaft mbH

POLICIES
Institute for Economic
and Innovation Research

Leonhardstrasse 59
8010 Graz, Austria

policies@joanneum.at
www.joanneum.at/policies

3
Medical University of Graz

Clinic of Internal Medicine
Division of Endocrinology and
Metabolism

Graz, Austria

Objective

Several tools exist to process the huge amount of data which are produced by untargeted metabolomics. However, the parameter settings for these tools significantly influence the reliability of the result. Here we present a parameter optimization approach to increase the reliability of the result produced by the open source package XCMS.

Methods

50 MCF-7 cell line samples were processed (Fauland et al. 2011) and mixed together to generate a pooled sample. This sample was injected periodically after every third sample into a LC-HRMS device (UHPLC-QExactive) (Fauland et al. 2011). Five of these measurements were then used for the optimization process.

The reliability of the peak picking process was assessed by using natural stable ^{13}C isotopes. Peaks belonging to an isotopologue were classified as **R**eliable **P**eaks (**RP**). If the estimated intensity of a ^{13}C isotope was at the lower limit of quantification, its ^{12}C peak was defined as **L**ow **I**ntensity **P**eaks (**LIP**). The **P**eak **P**icking **S**core (**PPS**) was calculated by:

$$PPS = \frac{\#RP^{1.5}}{\#all\_peaks - \#LIP}$$

The grouping step was needed for the evaluation of the retention time correction result. Therefore retention time correction and grouping were optimized simultaneously. The inverse of the average retention time deviations within the features was defined as a **R**etention time **C**orrection **S**core (**RCS**). This RCS was then used to optimize the retention time correction parameter settings. To optimize grouping parameter settings, features containing exactly one peak from each pooled sample injection were classified as 'good' features, all other features were classified as 'bad' ones. The ratio of the 'good' to 'bad' features resulted in a **G**rouping **S**core (**GS**). Calculation of the Retention time correction and **G**rouping **T**arget **V**alue (**RGTV**) was done by:

$$RGTV = norm(RCS) + norm(GS)$$

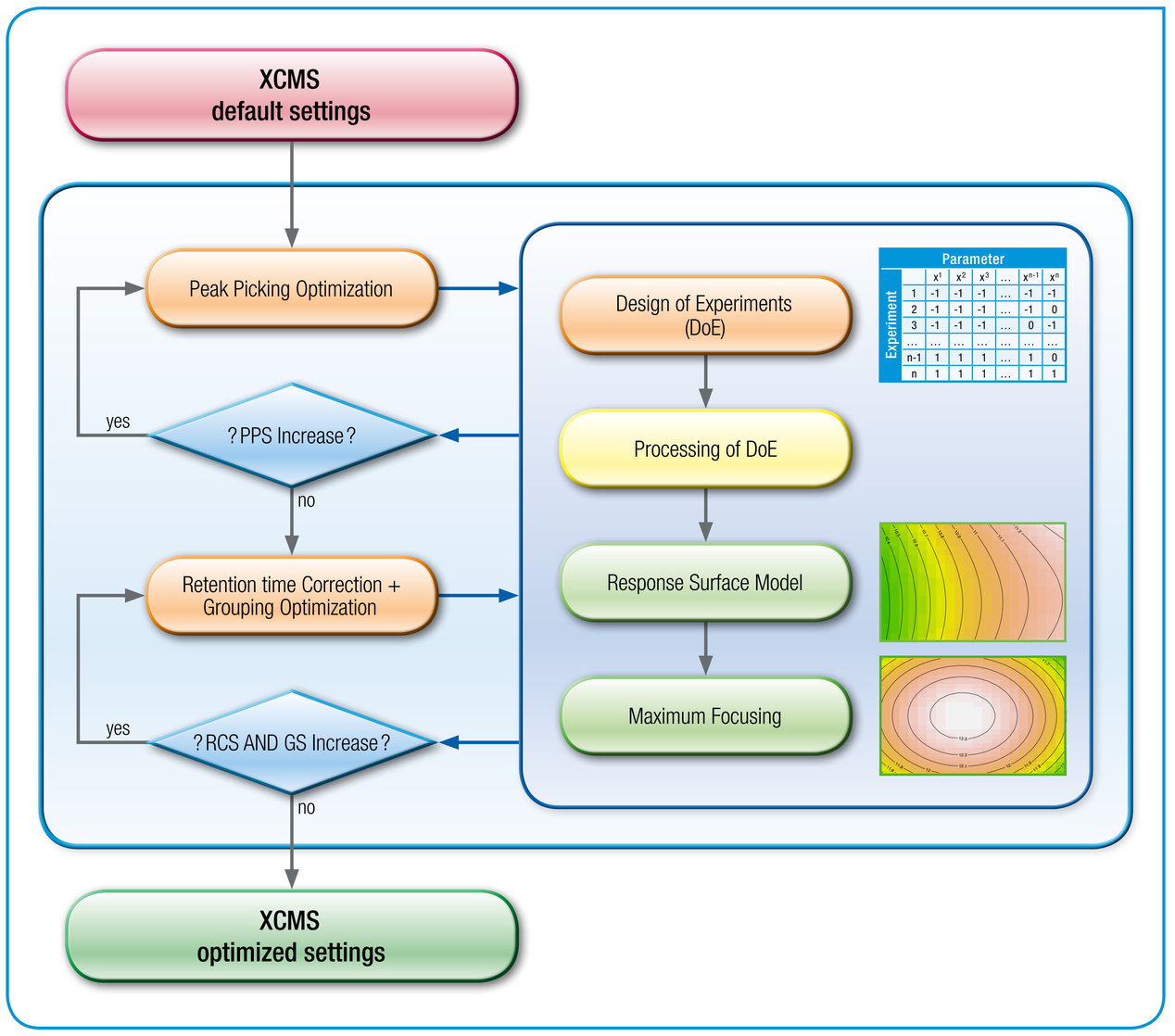

***Figure 1**: Workflow of the optimization process.*

***Table 1**: Comparison of default and optimized settings*

	Default settings	Optimized settings
# peaks	37,197	34,006
# RP [a]	11,263	11,682
# LIP [b]	17,718	17,274
PPS [c]	61.36	75.46
RCS [d]	55.36	482.84
good features	1,687	4,234
bad features	3,056	7
GS [e]	0.55	604.86

[a] Reliable Peaks; [b] Low Intensity Peaks; [c] Peak Picking Score; [d] Retention time Correction Score; [e] Grouping Score

Result

The total number of peaks decreased by 3,191 comparing the default and optimized settings. The number of reliable peaks was increased by 419. This led to a PPS increase of 23 %. The retention time deviations within the features were reduced to less than an eighth. The number of 'good' features increased by 2,547 and only 7 'bad' features appeared using the optimized settings compared to 3,056 achieved with the default settings.

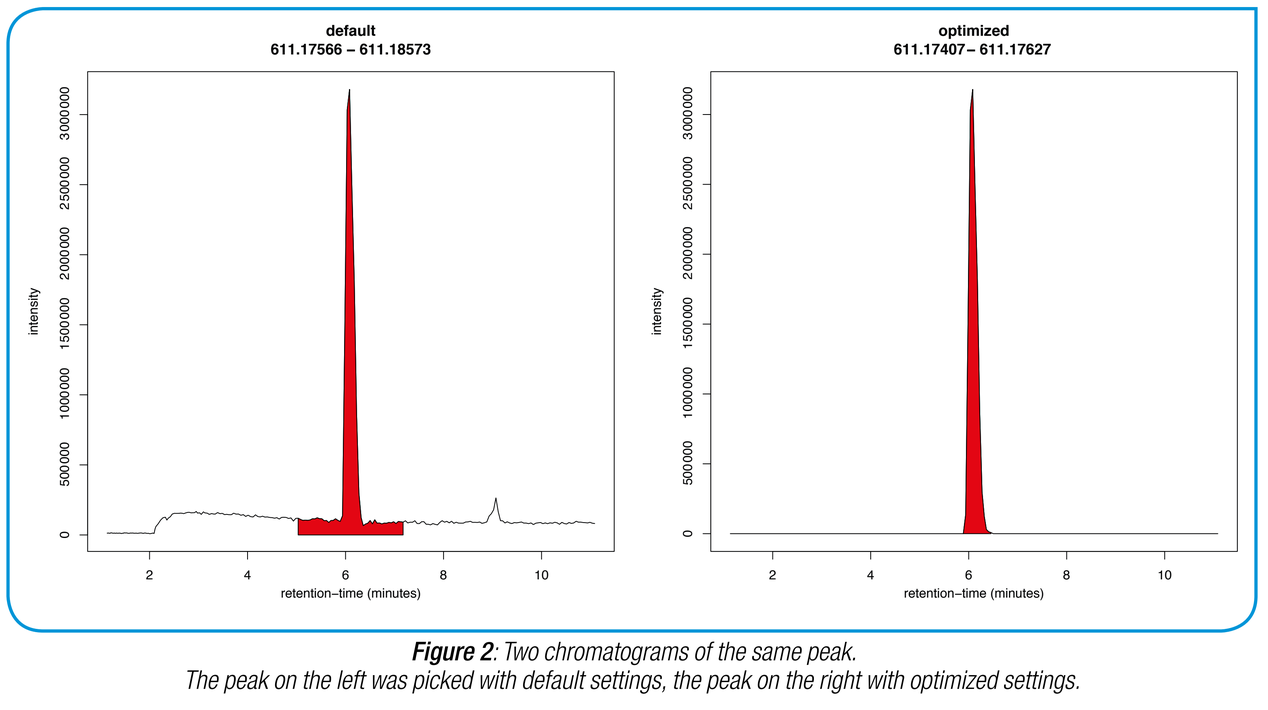

***Figure 2**: Two chromatograms of the same peak.*
The peak on the left was picked with default settings, the peak on the right with optimized settings.

Reference

Fauland A, Köfeler H, Trötzmüller M, et al. (2011) A comprehensive method for lipid profiling by liquid chromatography-ion cyclotron resonance mass spectrometry. J Lipid Res 52:2314–2322. doi: 10.1194/jlr.D016550

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