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REPORT

Liquid chromatography with accurate mass measurement on a triple quadrupole mass spectrometer for the identification and quantification of *N*-lactoyl ethanolamine in wine

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In this addendum to the original article [de Rijke, E., Ruisch, B.J., Bouter, N., König, T., Liquid chromatography with accurate mass measurement on a triple quadrupole mass-spectrometer for the identification and quantification of *N*-lactoyl ethanolamine in wine, *Mol. Nutr. Food Res.*, 2006, *50*, 351–355] a method is described to demonstrate that no potential cross-contamination from the reference target molecule had given rise to an incorrect positive identification of *N*-lactoyl ethanolamine in wine.

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1 Introduction

The Working Group on Methods of Analysis of the International Organisation of the Flavour Industry (IOFI) has developed and published a guideline to evaluate the validity of the identification of flavor molecules in nature [1]. This guideline was limited to GC-MS because most of the flavor molecules discovered until recently were sufficiently volatile to be analyzed by this technique.

As described in this publication in recent years more and more molecules of higher molecular weight or more polar character having taste or odour properties have been found in foodstuffs – hence, LC-MS is becoming more and more a routine technique in flavor research.

Therefore it became necessary for IOFI to develop an additional guideline for the use of LC-MS and define criteria for a valid identification.

One aspect of these criteria is that the scientist must ''ensure that no cross-contamination has given rise to a false positive'' by performing a ''blank'' experiment, or ''provide all necessary evidences that there was no possible contamination'' [see IOFI guidelines for LC-MS identifications in nature of flavouring substances, in this issue].

To have a practical example for the new LC-MS guideline, IOFI asked us to submit an addendum to our original publication [2] in which the aspect of ensuring those potential cross-contaminations is demonstrated.

Therefore we repeated the analytical approach as described in the original article.

2 Materials and methods

All materials and methods were the same as in the original article apart from the wine – the original one was not available any more. This time we chose Kracher Burgenland Beerenauslese Zweigelt. The only difference for the LC-MS/MS method was that it was performed in nominal mass mode instead of accurate mass mode for reasons of simplicity.

3 Results

Preparative LC fractionation: preparative LC-fractionation was performed in the same way as before (see original article), using a reference sample of pure *N*-Lactoyl ethanolamine to determine its retention time (6.2 min).

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Abbreviation: IOFI, International Organisation of the Flavour Industry

To show that no cross contamination occurred we applied the following protocol:

(i) Determination of the retention time of the target molecule for the isolation by preparative LC-ELSD by use of a 1% solution of *N*-lactoyl ethanolamine in water.
(ii) Cleaning the system by injecting water four times. Replacing the column by another identical one which had no contact with the reference material before. Cleaning the system again by performing four runs.
(iii) To test for the absence of cross contamination we injected 200 μL water eight times, and collected fractions between 5.7 and 6.7 min, which correspond to the elution time of the target. These fractions were pooled, concentrated to 0.5 mL (resulting in a concentration factor of 3.2), and subjected to LC-MS/MS (SRM) analysis.
(iv) Directly afterwards the wine was injected without any sample pre-treatment (200 μL) 35 times, the relevant fractions were collected, pooled, concentrated to 400 μL, and subjected to LC-MS/MS (SRM) analysis.
(v) Retention time changes were checked by collecting, pooling, and concentrating the fractions 1 min before and 1 min after the target fraction. This check was performed for both the blank and the wine.

In addition the LOD of *N*-lactoyl ethanolamine in the respective water solution was determined to be 3 μg/L. This is a significantly lower LOD compared with that of the SRM experiment described in the original article [2]. This time the SRM experiment was carried out in normal mass mode. Therefore additional lock masses for the accurate mass mode did not need to be included in the MS/MS experiment, which resulted in this lower LOD.

Figure 1 shows the LC-MS/MS chromatogram and the MS-MS results at the relevant retention time for *N*-lactoyl ethanolamine of the pooled and concentrated water injections. Obviously no *N*-lactoyl ethanolamine was detected, which means that the potential presence of cross contamination must be below the LOD of 0.9 μg/L (respecting the concentration factor of 3.2).

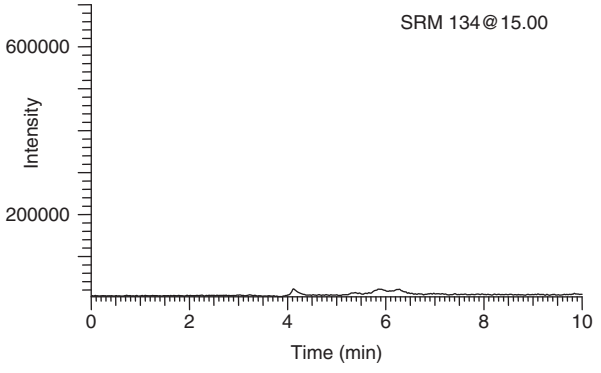

Figure 1. SRM chromatogram of *m/z* 134 of the combined and concentrated preparative LC ''blank'' fractions carried out before wine was injected into the system.

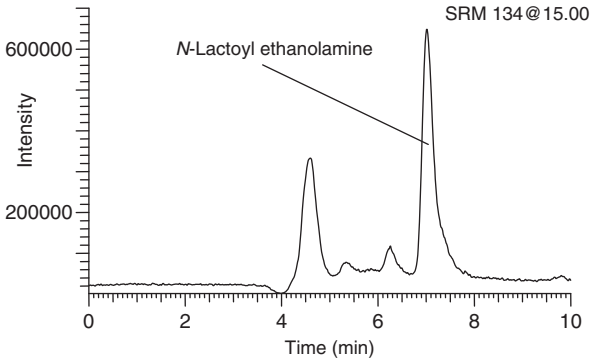

Figure 2. SRM chromatogram of *m/z* 134 of the combined and concentrated preparative LC ''wine'' fractions with positive identification of *N*-lactoyl ethanolamine.

In Fig. 2 the LC-MS/MS trace and MS-MS result (nominal mass measurement) of the relevant pooled and concentrated preparative LC fraction of the wine are given. The amount of *N*-lactoyl ethanolamine in the wine was determined to be 12 μg/L (external 3-point calibration, data not shown).

4 Discussion

Cross-contamination in the laboratory can be a source of incorrect positive identification of target analytes, especially at the trace level. Therefore it is good practice for an unambiguous identification that the analytical protocol contains a control step to ensure the absence of cross-contamination.

We were able to show by applying the same analytical investigation (LC-MS/MS) on the blank runs between working with the reference material and the sample under investigation (in this case the wine) that any doubt about the positive identification of *N*-lactoyl ethanolamine in wine can be ruled out.

We want to thank the colleagues from IOFI's Working Group on Methods of Analysis for the competent and fruitful discussion about the application of LC-MS/MS in flavor analysis.

The authors have declared no conflict of interest.

5 References

[1] Statement on the identification in nature of flavouring substances, made by the Working Group on Methods of Analysis of the International Organization of the Flavour Industry (IOFI). *Flavour Fragr. J.* 2006, *21*, 185.

[2] de Rijke, E., Ruisch, B. J., Bouter, N., König, T., Liquid chromatography with accurate mass measurement on a triple quadrupole mass-spectrometer for the identification and quantification of *N*-lactoyl ethanolamine in wine. *Mol. Nutr. Food Res.* 2006, *50*, 351–355.

