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Original Research Article

A strategy for validating concentrations of oxylipin standards for external calibration

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ABSTRACT

Quantitative analysis of oxylipins by means of chromatography/mass spectrometry is based on (external) calibration with standard compounds. Therefore, the quality of analytical standards is of fundamental importance for accurate results. Recently launched certified standards with an assured concentration within a narrow range are useful tools to verify analytical standards. However, such standards are only available for a few compounds.

Based on the exemplary comparison of certified with none certified standards we suggest a tiered approach to validate and control the concentrations when preparing an external calibration based on non-certified oxylipin standards. Concentrations are evaluated by means of liquid chromatography negative electrospray ionization mass spectrometry (LC-ESI(-)-MS) in selected ion monitoring mode and UV spectroscopy.

Based on the suggested approach, more than 50% of the standards in our calibration mix could be validated. Though most of the non-certified standards are of good quality, several oxylipin concentrations differ considerably demonstrating that a quality control strategy as suggested here is a mandatory prerequisite for quantitative oxylipin metabolomics.

1. Introduction

Quantitative analysis of oxylipins by means of chromatography/mass spectrometry is based on (external) calibration with standard compounds. Therefore, the quality of analytical standards is of fundamental importance for accurate results. We believe that differences in the used standard material cause the partly massively diverging oxylipin concentrations reported in literature e.g. for human plasma samples by different labs. This hypothesis is supported by our experience that differing results are also obtained from direct laboratory comparison when analyzing the same material.

The correctness of standard concentration needs to be ensured and/or experimentally validated and controlled. Recently launched certified standards with an assured concentration within a narrow range, e.g. MaxSpec standards (Cayman Chemical, Ann Arbor, MI, USA, purity ≥95%, tolerated inter-batch variation within 90–110% and the exact concentration is specified in the certificate of analysis), are useful tools for this purpose. However, such standards are only available for a few compounds at the moment and therefore do not directly serve to validate all of the 100–200 usually non-certified analytes commonly covered by targeted oxylipin metabolomics [1].

In order to overcome this limitation and be able to characterize and validate quality and concentration of a great number of analytes, we here suggest a tiered approach utilizing different analytical techniques, i.e. liquid chromatography-mass spectrometry (LC–MS) and UV spectroscopy.

2. Material and methods

All non-certified standards in our calibration mix were measured together with the few certified ones (100 nM in methanol) by means of LC–MS on a QTRAP (SCIEX, Darmstadt, Germany) QqQ MS instrument, as established in our lab described in detail elsewhere [2]. The peak areas in selected ion monitoring mode (SIM) were compared. UV absorption was evaluated in solution of individual compounds (300 µM in ethanol) using a microplate of a Tecan Infinte M Plex Reader (Tecan

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DiHDPE, dihydroxy docosapenatenoic acid; DiHETrE, dihydroxy eicosatrienonic acid; ESI, electrospray ionization; FA, fatty acid; HDHA, hydroxy docosahexaenoic acid; HETE, hydroxy eicosatetraenoic acid; LC, liquid chromatography; MS, mass spectrometry; PUFA, polyunsaturated fatty acid; RT, retention time; SIM, single ion monitoring; UV, ultraviolet

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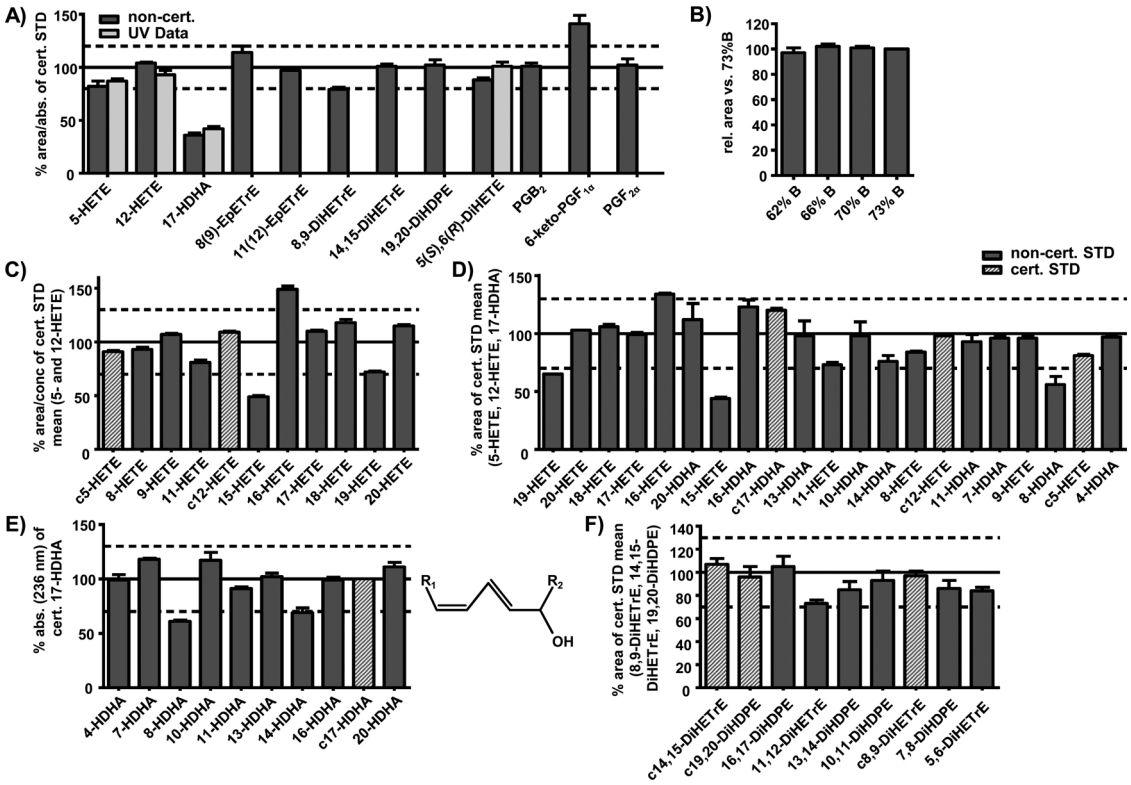

Fig. 1. Evaluation of purity of oxylipin standards (STD) by means of LC-ESI(-)-MS and UV absorption (Hydroxy-PUFA: 236 nm; 5(*S*),6(*R*)-DiHETE: 273 nm).
A) Comparison of certified (cert.) STD to non-certified STD: Selected ion monitoring (SIM) areas of non-certified STD (100 nM) are shown relatively (%) to the corresponding certified analyte. The accepted range of ± 20% is depicted by dashed lines.
B) Influence of mobile phase composition on SIM area of 5-HETE during the retention time window of ARA-derived hydroxy-PUFA, (18.00–21.80 min, 62–72%B).
C) Comparison of regioisomers of hydroxy-PUFA: The SIM areas of ARA-derived hydroxy-PUFA (100 nM) are depicted relative to those of certified 5- and 12-HETE STD (cross-hatched). The accepted range of ± 30% is depicted by dashed lines.
D) Comparison of hydroxy-FA from different PUFA: The SIM areas of ARA and DHA-derived hydroxy-PUFA (100 nM) are depicted relative to those of certified 5-HETE, 12-HETE and 17-HDHA STD (cross-hatched), analytes are sorted by retention time (RT). The accepted range of ± 30% is indicated by dashed lines.
E) UV absorbance (236 nm) of regioisomers of DHA- derived hydroxy-PUFA (300 µM). The chromophore of all analytes (1*Z*, 3*E*-pentadien system) is presented on the right. Shown is the blank corrected absorption relative to the certified 17-HDHA STD. The accepted range of ± 30% is depicted by dashed lines.
F) Comparison of dihydroxy-FA from different PUFA: The SIM areas of ARA- and DHA-derived dihydroxy-PUFA (100 nM) are depicted relative to those of certified 8,9-DiHETrE, 14,15-DiHETrE and19,20-DiHDPE STD (cross-hatched), analytes are sorted by RT. The accepted range of ± 30% is indicated by dashed lines.
All results are shown as mean ± SD (n = 3).

Austria GmbH, Grädig, Austria). All standards were obtained from Cayman Chemical, Ann Arbor, MI, USA and LC–MS grade acetic acid, acetonitrile and methanol were from Fisher Scientific (Schwerte, Germany).

3. Results

The SIM areas of non-certified standards were directly compared to the SIM areas of their corresponding certified standards in case they are also available in higher quality. And if there was no corresponding certified standard available the areas were compared to regiosomers or oxylipins from the same class from different precursor PUFA. UV absorption was also compared in case the compounds contained a structural UV-absorbing moiety and results from both analyses are shown in Fig. 1.

4. Discussion

For most compounds good comparability between certified and non-certified standards was observed, e.g. 5-HETE, 5(*S*),6(*R*)-DiHETE or $PGF_{2\alpha}$ which were within a range that is acceptable for us of ± 20% of the certified standard's area (Fig. 1A). For other oxylipins the differences between both standards were huge (≥60%), e.g. 17-HDHA or 6-keto-$PGF_{1\alpha}$ (Fig. 1A). UV absorption revealed the same differences between the certified and non-certified material, supporting the results obtained from LC–MS analyses. Moreover, robustness of the approach was investigated by conducting independent LC–MS analyses on different systems in two different labs (Sciex QTrap 6500 and 5500, Supplemental Fig. S1).

For evaluation of standards with no corresponding certified material available we directly compared the MS signal and UV absorption to regioisomeric certified standards. Under isocratic conditions the same response in SIM can be assumed among regioisomers. However, analysis of a large number of structurally different oxylipins requires the use of solvent gradients, influencing the ionization procedure. Though, with a shallow gradient (± 11%B, i.e. organic solvent: 800/150/1 (v/v/v) acetonitrile/methanol/acetic acid [2]) the SIM area of ARA-derived hydroxy-fatty acids (HETEs) is not affected as shown exemplarily for 5-HETE (Fig. 1B). Thus we concluded that our standard gradient for oxylipin analysis [2] can be used during validation of standards in SIM-mode: Comparing SIM areas of HETE regioisomers with the mean area of the two certified standards 5- and 12-HETE revealed major differences (> ± 30%) for 15- and 16-HETE (Fig. 1C), and precise correction factors based on the certified HETE-standards can be determined and implemented for analysis of samples.

This approach is not only limited to regioisomers derived from the same precursor FA, but also can be used for oxylipins from the same class, e.g. hydroxy-FA (Fig. 1D). Similar results for HETEs were

obtained when SIM areas of ARA- and DHA-derived hydroxy-FA were compared. Here, the SIM signal of one non-certified standard, i.e. 8-HDHA lied out of the accepted range of ± 30%. In order to substantiate the results of the SIM area approach, ideally, UV absorption (236 nm) can be used for the evaluation of most hydroxy-FA because they contain the same conjugated electron system (1*Z*, 3*E*-pentadien system). Based on this, a comparable molar absorption coefficient can be expected and the absorption can be directly compared (Fig. 1E). The approach can be adapted to other classes of oxylipins grouped together based on their chemical structure, such as dihydroxy-FA, exemplarily shown for all ARA- and DHA-derived dihydroxy-FA (Fig. 1F).

Conclusively, comparison of SIM areas in LC-ESI(-)-MS has proven to be a helpful tool for evaluating the quality of non-certified standards. Whenever possible, UV absorption should be considered to additionally confirm the results from LC–MS measurements.

Based on these results, we suggest the following strategy to validate (multi-analyte) oxylipin standard series since only a limited number of certified material is currently available:

1) High quality standards (with tight quality specifications) should be used for direct comparison of the corresponding non-certified standards included in (multianalyte) standard series. SIM areas/UV absorption must be within a ± 20% range of the certified standard's, otherwise factors for correction of the non-certified standard concentration should be implemented in the calibration. The ± 20% range was arbitrarily chosen based on the analytical variation of oxylipin levels for human plasma samples which is generally below 20% (in most cases 5–15%) [3].
2) Compounds with no corresponding certified standards available should be evaluated by comparing SIM areas/UV absorption to regioisomeric certified-standards. Regular gradient methods which are commonly used in targeted oxylipin metabolomics can be applied. If the SIM areas are within a range of ± 30% of the certified standard's, the concentration of the non-certified standard is acceptable. Otherwise, correction factors for these compounds based on the regioisomeric certified standard's SIM area are determined.
3) For analytes with no corresponding or regioisomeric certified standards at hand, precursor independent comparison of oxylipins from the same class (e.g. hydroxy- or dihydroxy-FA) can be carried out. Here, ratios are calculated relative to the (mean) SIM areas of certified standards from the same class. The same procedure is done for UV data, comparing certified standards with oxylipins containing the same structural absorbing moiety. Again, for compounds whose relative SIM area/UV absorption exceeds a limit of ± 30% of the certified standard's, correction factors based on the certified standard's SIM area are determined via LC–MS.

Not all standards commonly used in targeted oxylipin metabolomics methods can be evaluated by this approach, because no certified standards are available for all oxylipin classes (e.g. trihydroxy-FA) and some oxylipin classes are composed of structurally diverse compounds with insufficient UV absorption (mainly prostanoids and isoprostanes), and thus, comparison of SIM area and/or UV absorption is hampered. An extended portfolio of certified standards particularly for multiple hydroxylated-FA and isoprostanes is urgently required which would allow validation of the concentration of these oxylipin classes.

5. Conclusion

Based on the suggested approach, more than 50% of the standards in our calibration mix could be validated. The results show that most of the non-certified standards are of good quality. Their concentration is indicated correctly and in good accordance with the certified material. However, in some cases concentrations of non-certified standards can differ considerably from the nominal concentration. Therefore, a validation strategy as suggested here and calculation of correction factors in order to adjust concentrations and compensate for the differences is a prerequisite for accurate quantification of oxylipins.

Nevertheless, more certified standards are needed in order to be able to validate the concentration of all oxylipin classes, especially multiple hydroxylated-FA and the structurally diverse isoprostanes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prostaglandins.2019.02.006.

References

[1] I. Willenberg, A.I. Ostermann, N.H. Schebb, Targeted metabolomics of the arachidonic acid cascade: current state and challenges of LC–MS analysis of oxylipins, Anal. Bioanal. Chem. 407 (2015) 2675–2683.
[2] K.M. Rund, A.I. Ostermann, L. Kutzner, J.-M. Galano, C. Oger, C. Vigor, S. Wecklein, N. Seiwert, T. Durand, N.H. Schebb, Development of an LC-ESI(-)-MS/MS method for the simultaneous quantification of 35 isoprostanes and isofurans derived from the major n3- and n6-PUFAs, Anal. Chim. Acta 1037 (2018) 63–74.
[3] A.I. Ostermann, T. Greupner, L. Kutzner, N.M. Hartung, A. Hahn, J.P. Schuchardt, N.H. Schebb, Intra-individual variance of human plasma oxylipin pattern: low inter-day variability in fastened blood samples versus high variability during the day, Anal. Methods 10 (2018) 4935–4944.