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Recommendation for large-scale global lipidome analysis

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Letter to the Editor

Shotgun lipidomic analysis of chemically sulfated sterols compromises analytical sensitivity: Recommendation for large-scale global lipidome analysis

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Shotgun lipidomics affords comprehensive and quantitative analysis of lipid species in cells and tissues at high-throughput [1–5]. The methodology is based on direct infusion of lipid extracts by electrospray ionization (ESI) combined with tandem mass spectrometry (MS/MS) and/or high resolution Fourier transform mass spectrometry (FTMS) for identification and quantification of lipid species [6]. Shotgun lipidomics affords extensive lipidome coverage by combining the analysis of lipid extracts in positive and negative ion mode [1, 3]. Notably, sterols such as cholesterol and ergosterol exhibit low ionization efficiency in ESI [7]. For this reason, chemical derivatization procedures including acetylation [8] or sulfation [9] are commonly implemented to facilitate ionization, detection and quantification of sterols for global lipidome analysis [1–3, 10].

In the course of large-scale lipidomic analyses using an LTQ Orbitrap XL mass spectrometer (Thermo Scientific) equipped with a robotic nanoESI source TriVersa NanoMate (Advion Biosciences), we observed a pronounced decrease in the sensitivity of negative ion mode analysis following repeated injections of samples subjected to chemical sulfation. This decrease in sensitivity was observed only for negative ion mode analysis of lipid species in underivatized lipid extracts. No decrease in sensitivity was observed for negative ion mode analysis of chemically sulfated sterols or for positive ion mode analysis of lipid species in underivatized lipid extracts.

To investigate the decrease in analytical sensitivity in further detail we (i) spiked a yeast lysate with internal standards and executed two-step lipid extraction as previously described [3].

### Abbreviations

- ESI, electrospray ionization; FTMS, Fourier transform mass spectrometry; MS/MS, tandem mass spectrometry; PI, phosphatidylinositol

For the partition of apolar and polar lipids, (ii) subjected the apolar lipid extract (containing sterols) to chemical sulfation [2, 9], and (iii) infused the sulfated sample in batches of ten repeated injections and monitored the intensity of chemically sulfated sterols by negative ion mode FTMS. To monitor the decrease in sensitivity we analyzed the underivatized polar lipid extract [containing phosphatidylinositol (PI) as well as other polar lipids [3]] by negative ion mode FTMS before and after each batch of ten repeated injections of the sulfated sample.

This analysis showed a progressive loss of sensitivity for negative ion mode analysis of lipid species in the underivatized polar lipid extract. To exemplify this effect, the intensity of PI 34:1, an abundant glycerophospholipid species in yeast [3, 10], was reduced more than 20-fold after 30 injections of the sulfated sample (Fig. 1a). More importantly, since an intensity threshold is routinely used in shotgun lipidomics data processing [11], the decrease in sensitivity translated into a decrease in the number of lipid species identified and quantified (data not shown). Notably, the intensity of chemically sulfated sterols was unaffected (Fig. 1b). As we had previously observed that the sensitivity of positive ion mode analysis was not affected, we transiently switched the polarity to positive for 1 h and subsequently reverted it to evaluate whether the sensitivity of negative ion mode analysis was restored. This polarity switch partially restored the sensitivity (>40%, in the case of PI 34:1) for negative ion mode analysis of lipid species in the underivatized polar lipid extract (Fig. 1a). Based on these results, we recommend that global shotgun lipidomic experiments employing chemical sulfation of sterols use a specific sequence of MS analyses where the analysis of sulfated samples is scheduled at the end of all other analyses in order not to compromise analytical sensitivity (Fig. 2).

In conclusion, here we: (i) demonstrate that shotgun lipidomic analysis of sample extracts subjected to chemical sulfation can prompt a pronounced loss of sensitivity for negative ion mode analysis of underivatized lipid extracts;
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