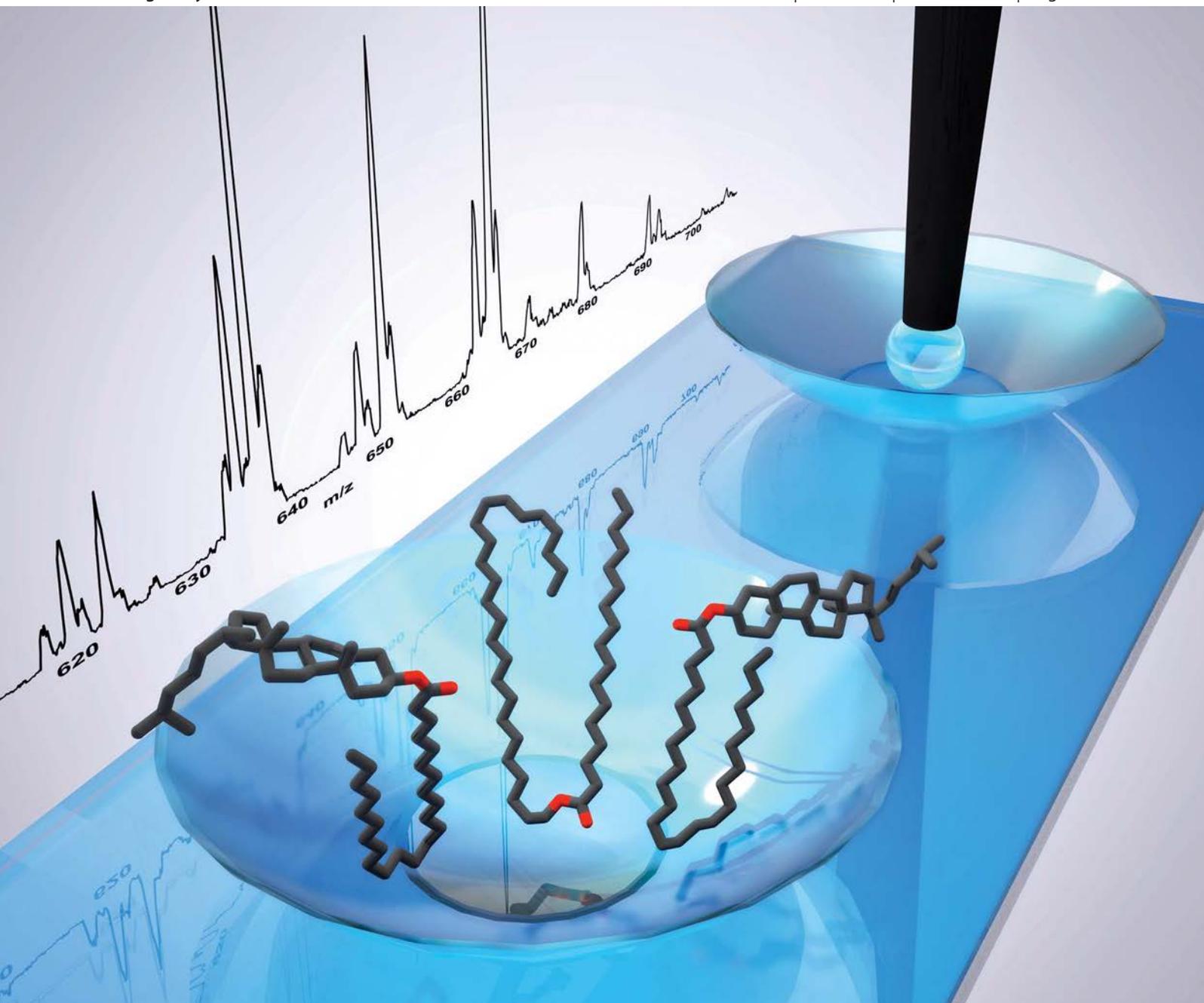


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**COMMUNICATION**

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## Automated surface sampling of lipids from worn contact lenses coupled with tandem mass spectrometry†

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The deposition of biological material (biofouling) onto polymeric contact lenses is thought to be a major contributor to lens discomfort and hence discontinuation of wear. We describe a method to characterize lipid deposits directly from worn contact lenses utilizing liquid extraction surface analysis coupled to tandem mass spectrometry (LESA-MS/MS). This technique effected facile and reproducible extraction of lipids from the contact lens surfaces and identified lipid molecular species representing all major classes present in human tear film. Our data show that LESA-MS/MS is a rapid and comprehensive technique for the characterization of lipid-related biofouling on polymer surfaces.

### Introduction

Contact lenses are a popular form of vision correction, with 140 million contact lens wearers comprising approximately 2% of the world population.<sup>1</sup> Commercially available since 1999, silicone hydrogel contact lenses were developed to improve oxygen permeability by inclusion of silicone and fluorine into traditional soft contact lens formulations, and have proven to substantially improve eye health.<sup>2</sup> These new hydrogel formulations have not solved the problem of accumulation of biological material as solid deposits on the surface of the lens polymer during lens wear. These deposits are primarily composed of lipids and protein adsorbed from the tear film and corneal surface.<sup>3,4</sup> Extensive studies have identified and quantified the protein components of these deposits, and recent reports demonstrated that the profiles of protein deposition are

dependent on contact lens formulation.<sup>3,5</sup> In contrast, limited information is available regarding the lipid composition of deposits on silicone hydrogel contact lenses.

The extent and speed of lipid biofouling has been quantified on silicone hydrogel contact lenses in both *in vitro* and *ex vivo* studies. *In vitro*, tear film lipids including cholesterol, cholesterol esters, and phospholipids accumulate on unworn silicone hydrogel contact lenses in a time dependant fashion.<sup>6,7</sup> *Ex vivo*, free cholesterol and phospholipids have been identified and quantified on a range on silicone hydrogel lens formulations.<sup>3,8</sup> Importantly however, recent characterization of the tear lipidome suggests that free cholesterol and phospholipids are only minor components of the endogenous tear film.<sup>9–11</sup> Consequently, new analyses of contact lenses are required to elucidate the complete molecular composition of lipid deposits.

Previous studies investigating lipid deposition on contact lenses have been performed using traditional mass spectrometric and biochemical techniques. While sensitive and comprehensive, these techniques are relatively time consuming and cannot discriminate between the discrete contact lens surfaces (*i.e.*, “air” or “eye” side). For example, traditional extraction of lipids from a contact lens requires bathing the lens in solvent, followed by bi-phasic extraction of the lipid.<sup>8</sup> This approach presents substantial analytical challenges. Most notably, solvents used to extract lipids can also remove polymer chains and additives, increasing the complexity and chemical noise in the analyte solution but also degrading the integrity of the lens itself such that it must be handled very carefully to prevent breakage. Therefore, a rapid technique for direct characterization of lipid-related biofouling on contact lens materials is attractive. An ideal technique would be suitable for comparison of deposits across a range of contact lens materials; give a detailed molecular profile of lipid build-up in clinical wear situations; and be sensitive enough to assess the efficacy of cleaning solutions in modulating biofouling. Herein we describe a method to analyze lipids directly from a contact lens utilizing liquid extraction surface analysis coupled to tandem mass spectrometry (LESA-MS/MS). Originally developed by the Van Berkel group, liquid extraction surface analysis (LESA) effects surface extraction by the creation of a

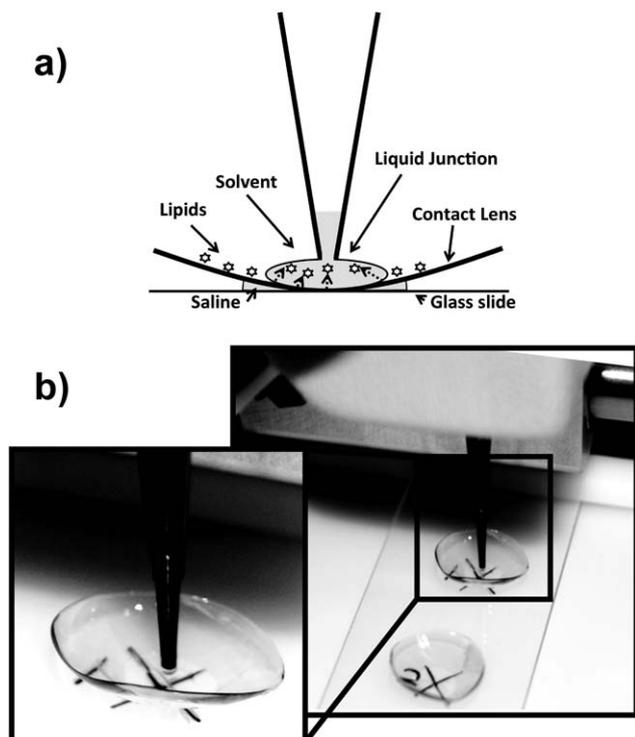
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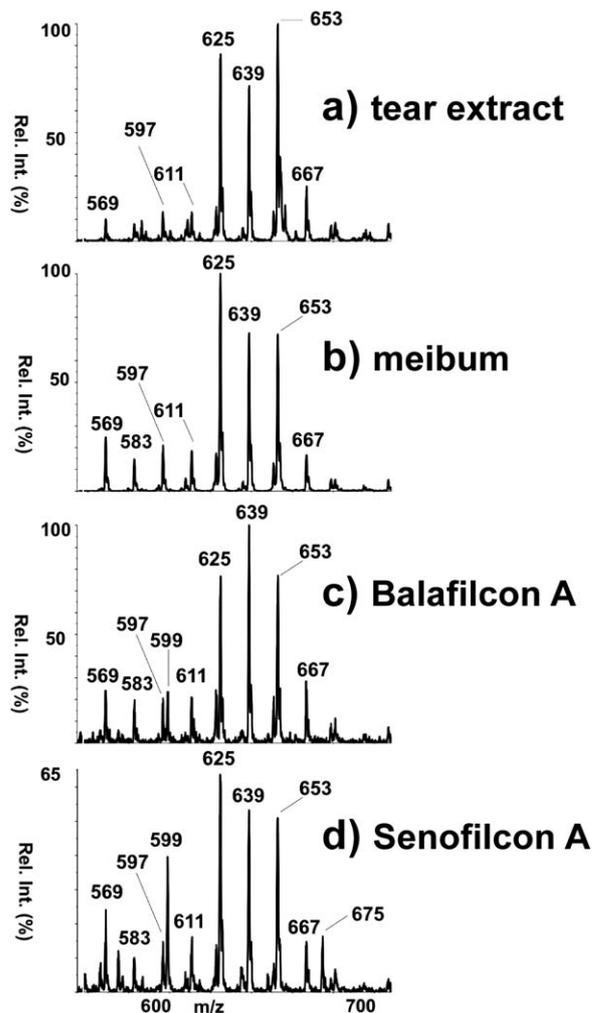
**Fig. 1** (a) Schematic diagram of LESA from a contact lens. Solvent was dispensed onto the contact lens from the pipette tip at a height of approximately 0.4 mm above the contact lens surface. A liquid junction was maintained between the pipette tip and the surface throughout the extraction. (b) Photograph of the LESA process. The solvent drop and liquid junction can be seen between the pipette tip and the contact lens.

liquid-surface microjunction between a small volume of solvent at the analytical surface and a pipette tip.<sup>12</sup> Following analyte extraction, the solvent is aspirated into the pipette tip and infused into the mass analyser *via* chip-based nanoelectrospray ionisation (nano-ESI). A sample-handling robot performs all extraction manipulations allowing LESA to be fully automated and reproducible. A photograph and schematic diagram of the tip and liquid junction is shown in Fig. 1.

## Results and discussion

Worn contact lenses were placed on a glass slide and the surface to be analysed was gently dried under nitrogen. Saline solution remaining on the opposing (lower) surface allowed adhesion between the contact lens and glass slide. LESA was performed on the exposed upper surface of the contact lens. Human tear and meibum samples were dissolved in chloroform, and a small aliquot of each were applied to the surface of a polytetrafluoroethylene (Teflon) printed slide.

LESA-MS/MS spectra were obtained from (a) human tear, (b) human meibum, (c) worn Balafilcon A contact lenses, and (d) worn Senofilcon A contact lenses. Representative precursor-ion mass spectra for ionized lipids that dissociate to form the protonated 17:0 fatty acid at a mass-to-charge ratio ( $m/z$ ) of 271 are shown in Fig. 2. While unusual in most animal tissues, lipids containing odd-chain fatty acids have been identified in both tears and meibum, and



**Fig. 2** MS/MS precursor ion scan targeting  $m/z$  271 ( $[17:0 \text{ fatty acid} + \text{H}]^+$ ) from LESA of: (a) human tear spotted on a Teflon coated glass slide, (b) human meibum spotted on a Teflon coated glass slide, (c) a worn Balafilcon A contact lens, (d) a worn Senofilcon A contact lens. Ions labeled at  $m/z$  569, 583, 597, 611, 625, 639, 653 and 667 were identified by subsequent CID as  $[\text{M} + \text{NH}_4]^+$  wax ester species containing a 17:0 fatty acid.

potentially may be more diagnostic for the unique biology of the meibomian gland. Peaks observed at  $m/z$  569, 583, 597, 611, 625, 639, 653, and 667 are consistent with  $[\text{M} + \text{NH}_4]^+$  ions from wax ester lipids: an assignment confirmed by subsequent collision-induced dissociation (CID) experiments, shown in Fig. S1.<sup>†</sup> The combination of these tandem spectrometry analyses identified the wax esters WE (17:0/20:0), WE (17:0/21:0), WE (17:0/22:0), WE (17:0/23:0), WE (17:0/24:0), WE (17:0/25:0), WE (17:0/26:0), WE (17:0/27:0), respectively. The ion observed at  $m/z$  599 does not match any known masses for 17:0 fatty acid WE, and was identified as a contaminant from the LESA extraction as it was observed when an unworn lens was subjected to the same analysis, shown in Fig. S2.<sup>†</sup> Although the signal-to-noise ratio was reduced in the spectra obtained directly from contact lenses (Fig. 2c and d) relative to the tear and meibum samples, the majority of lipids present in the latter were also detected in the contact lens analyses. LESA-MS/MS analysis of the tear and meibum samples provided representative profiles of all

significant classes of non-polar (wax and cholesterol esters) and polar lipids (phospholipids and *O*-acyl  $\omega$ -hydroxy fatty acids) previously identified.<sup>10</sup> Importantly, the analogous profiles across all lipid classes were also recovered upon direct analysis of worn contact lenses of both types examined namely, Balafilcon A and Senofilcon A. Representative spectra for these major classes are shown in Fig. S3.† Within each class of lipid, molecular speciation was comparable between the four sample types.

To demonstrate the analytical capabilities of LESA-MS/MS, lipid characterization was conducted on 25 worn contact lenses. A comparison of the cholesterol ester (CE) profiles obtained from the Balafilcon A and Senofilcon A lenses is shown in Fig. 3(a). A total of 42 CE species were identified with esterified acyl chains ranging from 14 to 34 carbons in length. Only the 20 most abundant species are shown for simplicity. Although the CE profiles were remarkably similar, some differences in abundance were observed between the two types of contact lenses. The most obvious difference was an increase in the contribution of the 16 carbon esters in the Balafilcon A extract, however the reason for this specific increase is unclear.

Prior attempts to characterise lipids deposits on contact lenses,<sup>8</sup> have relied on bulk extraction of soluble compounds from the lens material, which does not discriminate between the two surfaces of the lens. A significant advantage of LESA in this application is the discrete analysis of the pre- and post-contact lens surfaces (*i.e.*, “air side” or “eye side”). Fig. 3(b) shows a comparison between the CE profiles of pre- and post-surfaces contact lens surfaces. Notably, both surfaces had similar CE composition in lipid deposits, as might be expected. It should be noted that although analysis can be performed on either surface, the lens has been mounted *via* the alternate face so analysis of both sides of the same lens was not possible. While absolute quantification remains an ongoing

challenge in most forms of direct analysis mass spectrometry,<sup>14</sup> relative quantification within a lipid class by LESA-MS/MS was demonstrated without the use of internal standards.

Liquid extraction surface analysis has demonstrated considerable advantages for analysis of contact lens lipid deposition. This technique has proven to be a simple, rapid and comprehensive technique to extract lipid deposits from worn contact lenses, capable of extracting the majority of known lipid classes with limited degradation of the lens material. The flexibility of extraction contact time and extraction solvent allowed for rapid optimization of parameters for lipid desorption.

When coupled to a sensitive tandem mass spectrometer, in approximately fifteen minutes both extraction and mass spectrometric data acquisition were completed. In each sample, the molecular speciation of wax esters, cholesterol esters, phospholipids, and *O*-acyl- $\omega$ -hydroxy fatty acids was identified. These classes represent the major species reported in both tears and meibum.<sup>9,10,15,16</sup> Automation of both liquid extraction and mass spectrometric analysis provided for extraordinary consistency in what are often challenging samples. Our data show that LESA-MS/MS is efficient in the extraction and analysis of biofouling on polymeric materials, capable of extracting and determining the molecular composition of biological deposits with limited degradation of the underlying material.

## Experimental

### Sample collection and preparation

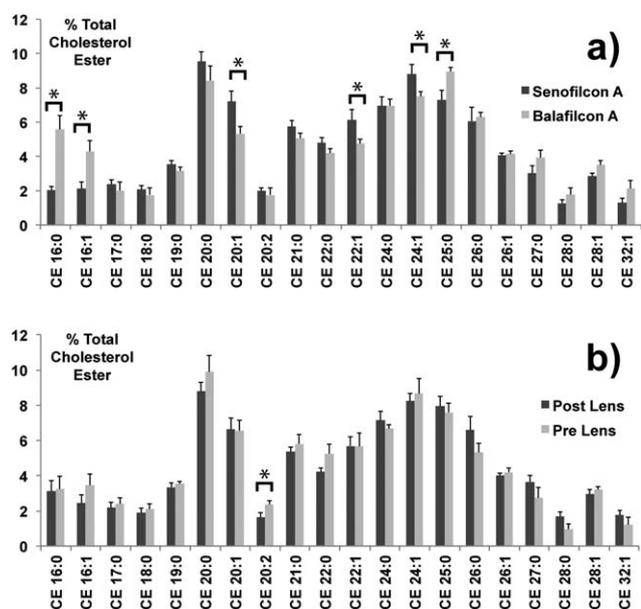
Twenty five contact lenses were collected from healthy individuals as described in Saville *et al.*<sup>8</sup> Two types of commercially available contact lenses, Senofilcon A (polyvinyl pyrrolidone) and Balafilcon A (*N*-vinyl pyrrolidone) were collected. The contact lenses were removed with sterile gloves, rinsed with a commercially available saline solution and stored at  $-80^{\circ}\text{C}$ .

In preparation for LESA, contact lenses were removed from storage containers using tweezers and placed on glass microscope slides in the LESA sample holder. Nitrile gloves were used when handling all microscope slides and contact lenses.

Excess liquid on the contact lenses was shaken off before the lenses were placed on the slide. Lenses subjected to pre-lens analysis were inverted before being placed on the slide. A flat area on the surface of the contact lens was created by liquid surface adhesion between the underside of the contact lens and the glass microscope slide. The analytical surface was dried with a focused flow of nitrogen gas before being allowed to dry for a further 10–15 minutes in air.

### Tears and meibum – collection and deposition for analysis

Patients gave informed consent in accordance with the Tenets of Helsinki before collection of tear or meibum sample. Meibum was collected from a single volunteer by the methods described in Saville *et al.*,<sup>17</sup> with the exception that chloroform was added to the vial prior to collection. In brief, meibum was expressed using a cotton bud to squeeze the eyelid, collected with a metal spatula, and stored in chloroform. Basal tear fluid was collected from a single volunteer by glass capillary at the lower lid margin with care to prevent



**Fig. 3** Profiles of the 20 most abundant cholesterol ester species identified by LESA-MS/MS from worn contact lenses, normalized to total cholesterol ester. (a) Comparison between profiles obtained from Senofilcon A ( $n = 17$ ) and Balafilcon A ( $n = 8$ ) contact lenses materials. (b) Comparison between profiles obtained from the post-contact lens ( $n = 15$ ) and pre-contacts lens ( $n = 10$ ) surfaces. \* $p < 0.05$ .

stimulating reflex tears. The tear sample was centrifuged in a polypropylene microcentrifuge tube at 10 000g for 10 minutes at 4 °C to remove cellular debris. The tear sample was then placed in glass vial and stored at –80 °C until analysis.

The meibum sample (50 µL) was added to 50 µL of chloroform containing 2.4 µM PC 17:0/17:0 and 2.4 µM dihydrosphingomyelin 12:0. Three µL of the meibum : solvent mixture was spotted onto a polytetrafluoroethylene (PTFE) printed slide (Prosolia Inc, Indianapolis, IN, USA) and dried in ambient air. The tear sample (~10 µL) was added to 50 µL of the chloroform mix described above. Three µL of the tear : solvent mixture was spotted onto a PTFE printed slide and dried in ambient air.

### Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS)

The acquisition of positive and negative ion LESA-MS spectra were achieved using a TriVersa Nanomate® (Advion, Ithaca, NY, USA) coupled to a hybrid linear ion trap-triple quadrupole QTRAP® 5500 mass spectrometer (ABSCIEX, Foster City, CA, USA) with Analyst® 1.5.1 software (ABSCIEX) used for spectral acquisition.

The automated robotic pipette tip in the Tri-Versa Nanomate® (Advion) aspirated a total volume of 3 µL of solvent, with 1.2 µL dispensed onto the surface of the contact lens from a height of 1.0 mm above the glass slide. A liquid junction formed between the contact lens surface and the pipette tip enabled the extraction of analytes from the surface of the contact lens. Following a 5 second delay, solvent was aspirated from a height of 0.6 mm above the glass slide. Due to the thickness of the lens, both dispensation and aspiration height above the contact lens was approximately 0.6 mm lower than described above, *i.e.* dispensation at approximately 0.4 mm above the contact lens and aspiration with the pipette tip almost touching the contact lens. These heights were adjusted in the LESA software to ensure a liquid junction was maintained for the entire extraction. Each contact lens was sampled once at a central location, with a droplet of approximately 2–3 mm in diameter. In each experiment the total solvent volume of 3 µL was delivered into the mass spectrometer *via* a nano-ESI chip with an orifice diameter of 4.1 µm. The delivery gas was N<sub>2</sub> at a pressure of 1.0 psi. For positive ion mode extraction and acquisition isopropanol–methanol–chloroform (4 : 2 : 1 v/v/v) with 20 mM ammonium acetate was used with a spray voltage of 1.0 kV. For negative ion mode extraction and acquisition methanol–chloroform (2 : 1 v/v) with 8 mM ammonium acetate and a spray voltage of 1.4 kV was used. Solvent conditions were chosen for optimal extraction and ionization of lipid species.<sup>18</sup> In preliminary experiments utilizing extended and/or repeated extractions, full MS scans were dominated by a series of peaks with a spacing of 74 Th indicative of siloxane containing polymer. For optimal signal-to-noise a single, 5 second extraction was used.

LESA-MS/MS spectra were acquired on a QTRAP® 5500 by precursor-ion scanning. Target lipids, respective precursor ion scan fragment *m/z*, and CID energy are shown in Table 1. Typical experimental conditions for positive mode acquisition were a declustering potential of 100 V, entrance potential of 10 V and a scan rate of 200 *m/z* units per s. Negative mode acquisition parameters were a declustering potential of –300 V, entrance

**Table 1** Target lipid class, precursor ion experiment, and CID energy utilized for LESA-MS/MS identification of lipid deposits on worn contact lenses. Wax ester (WE), cholesterol ester (CE), phosphatidylcholine (PC), sphingomyelin (SM), and *O*-acyl- $\omega$ -hydroxy fatty acid (OAHFA) molecular lipid species were detected

Target lipid	Polarity	Ion	Fragment ion <i>m/z</i>	CID energy (eV)
WE (17:0 fatty acid) <sup>13</sup>	+	[M + NH <sub>4</sub> ] <sup>+</sup>	271.3	30
CE <sup>19</sup>	+	[M + NH <sub>4</sub> ] <sup>+</sup>	369.4	25
PC and SM <sup>20</sup>	+	[M + H] <sup>+</sup>	184.1	55
OAHFA (18:1 fatty acid) <sup>21</sup>	–	[M – H] <sup>–</sup>	281.3	35

potential –11 V and scan rate 200 *m/z* units per s. Mass spectra were averaged over a minimum of 50 scans. All mass spectra were normalized to the most abundant ion in the spectrum. Cholesterol ester data were analyzed with LipidView® (ABSCIEX) version 1.1, including identification, de-isotoping, smoothing and isotope correction.

### Statistical analysis

A Student's *T* test was used to compare CE profiles. *P* values <0.05 were considered significant.

### Acknowledgements

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