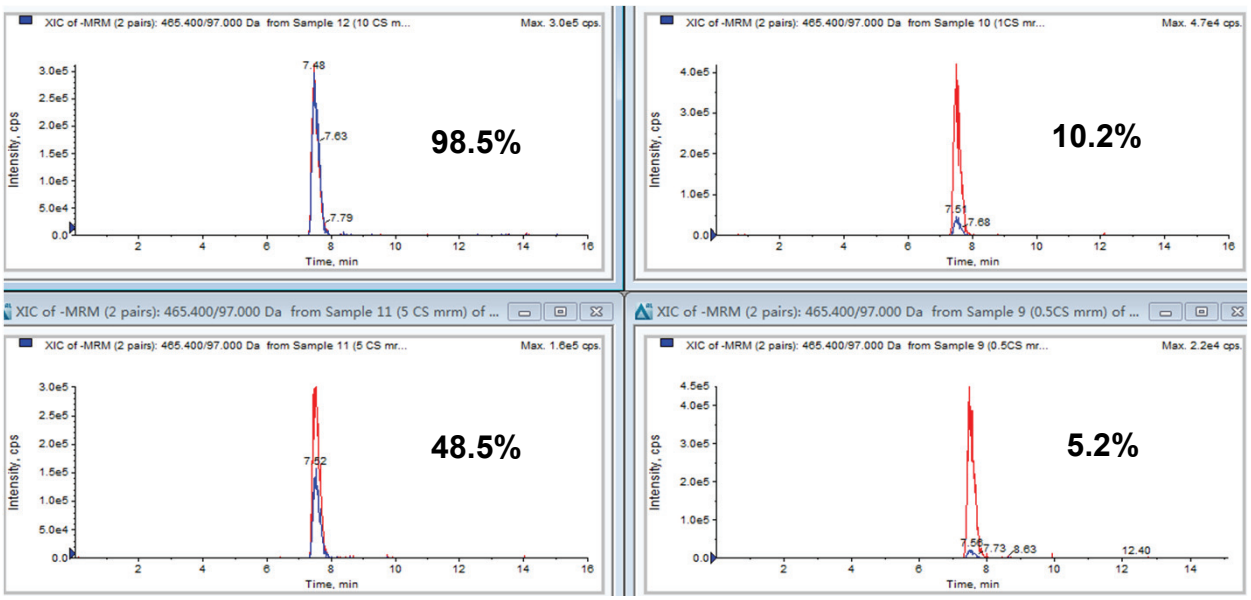
Figure S1, related to Figure 4. Validation of linearity of MRM method developed for quantitation of cholesteryl sulfates. Increasing concentrations of cholesteryl sulfates (0.5, 1.0, 5.0, 10.0 µg ml^{-1}) were spiked with equal volume of 10 µg ml^{-1} of d7-cholesteryl sulfate as internal standard and analysed individually under the chosen HPLC-RP-ESI-MRM conditions in the negative ion mode. **(A)** The extracted ion chromatograms for each concentration of cholesteryl sulfate used and **(B)** a plot of measured concentration against applied concentration were shown. The physiological concentrations of cholesteryl sulfates found in meibum and tear samples lie within the linearity range reported.

A



B

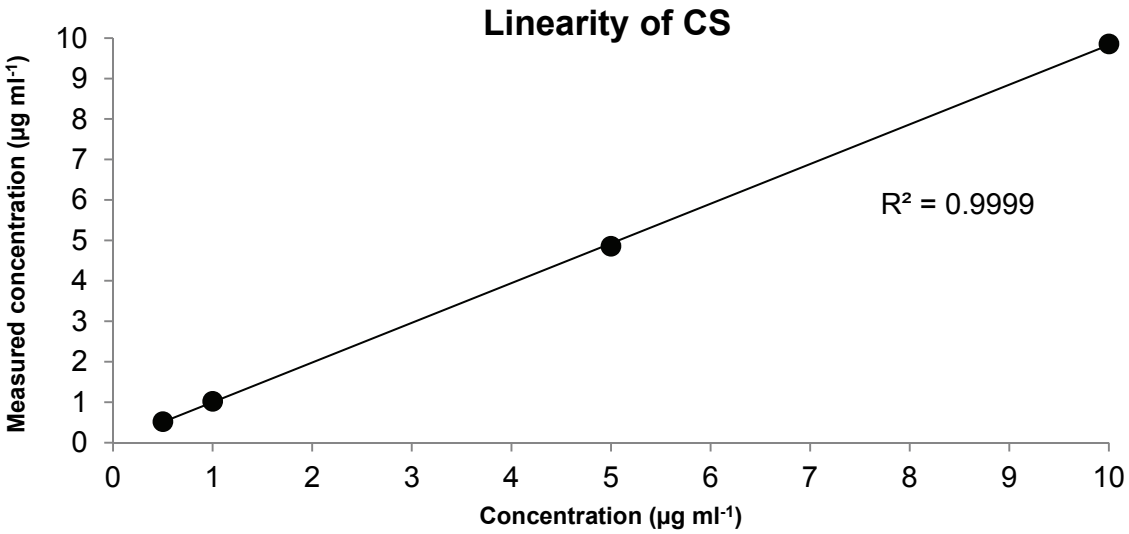


Figure S2, related to Figure 1. The distributions of major classes of nonpolar and polar lipids along the strips.

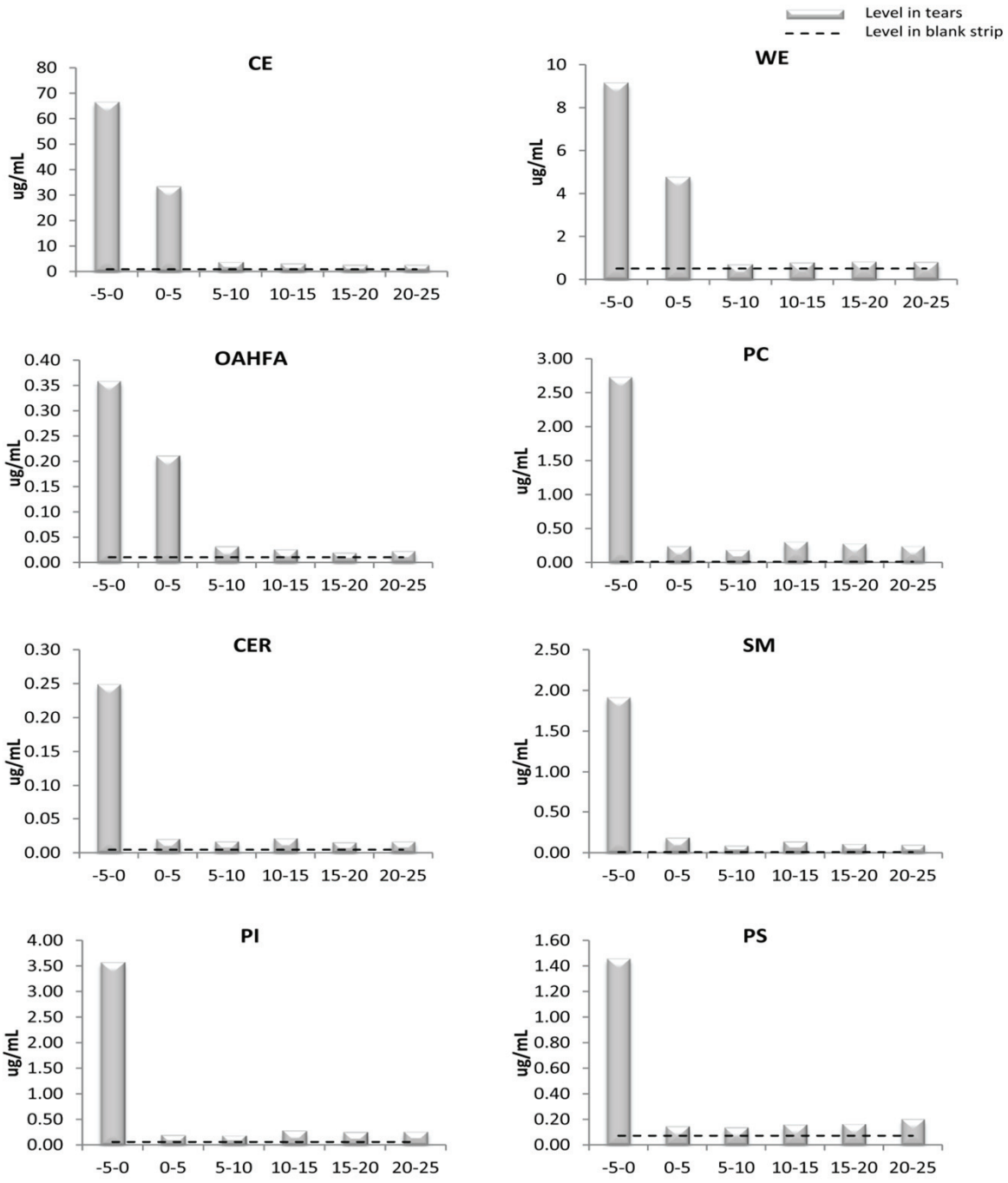


Figure S3, related to Figure 1. Plots of raw intensities against relative concentrations of lipid standards used.

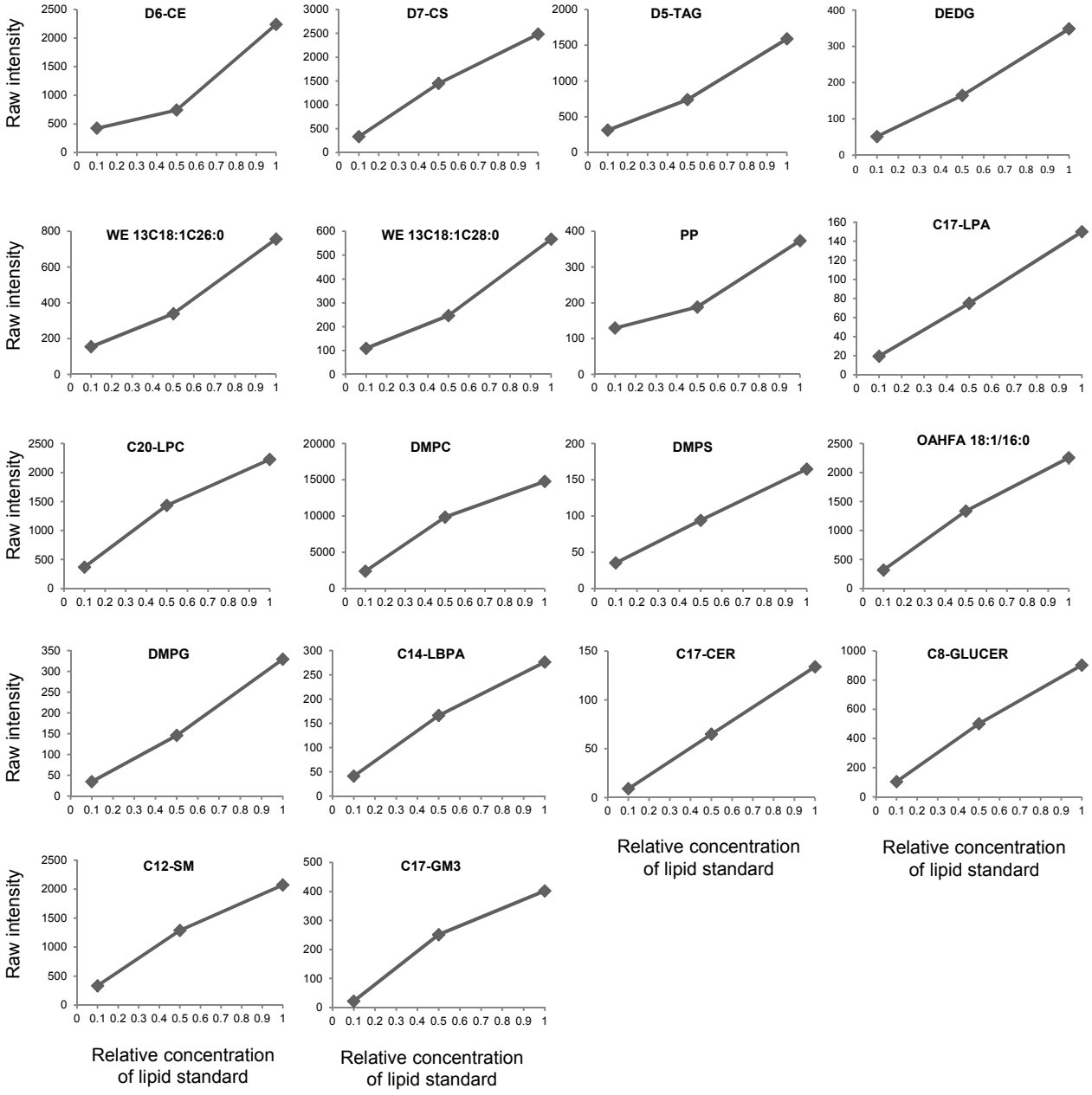


Figure S4, related to Figure 4. Single-stage mass spectra obtained using the Orbitrap. The ion spectra were obtained using single-stage scan with normal phase LC-separation in the positive mode for **(A)** SM and **(B)** PC and negative mode for **(C)** PE. Accurate masses were presented and only major species within each lipid class were shown.

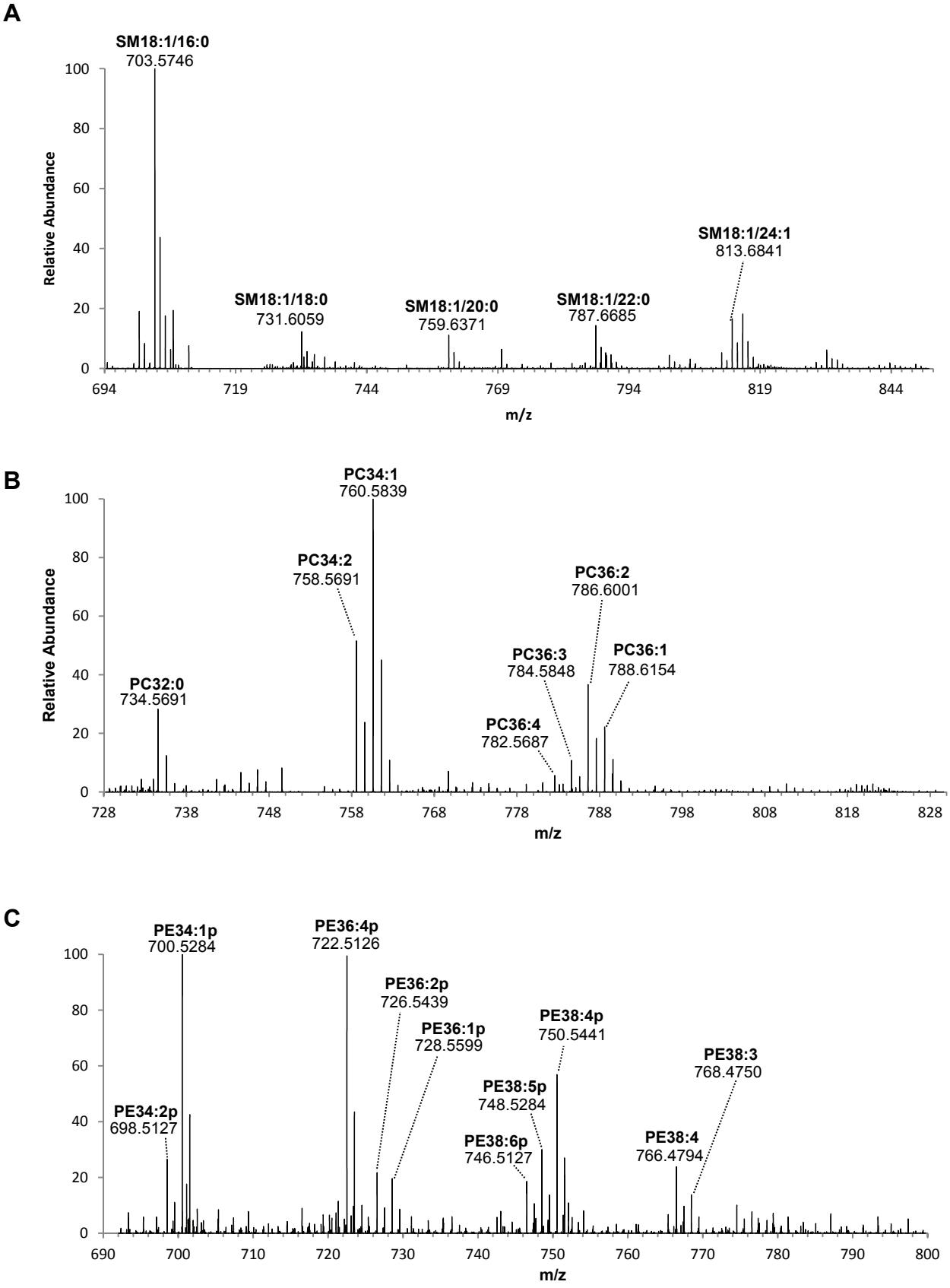


Figure S5, related to Figures 3-4. Heatmap illustrating the lipid profiles of all lipid species analyzed in 28 *individual* human tear samples from dry eye patients. Values were plotted as $\log_{10}$ of mole fraction of individual lipid species. It was observable that the lipid profiles were generally reproducible even in samples with very low Schirmer's score (<5mm). One anomalous sample of relatively high Schirmer's length displayed unusually low levels of polar lipids that might be related to disease pathology. The overall consistency in lipid profiles validated the sensitivity and reproducibility of our analytical approach even for samples with very low amount of tears.

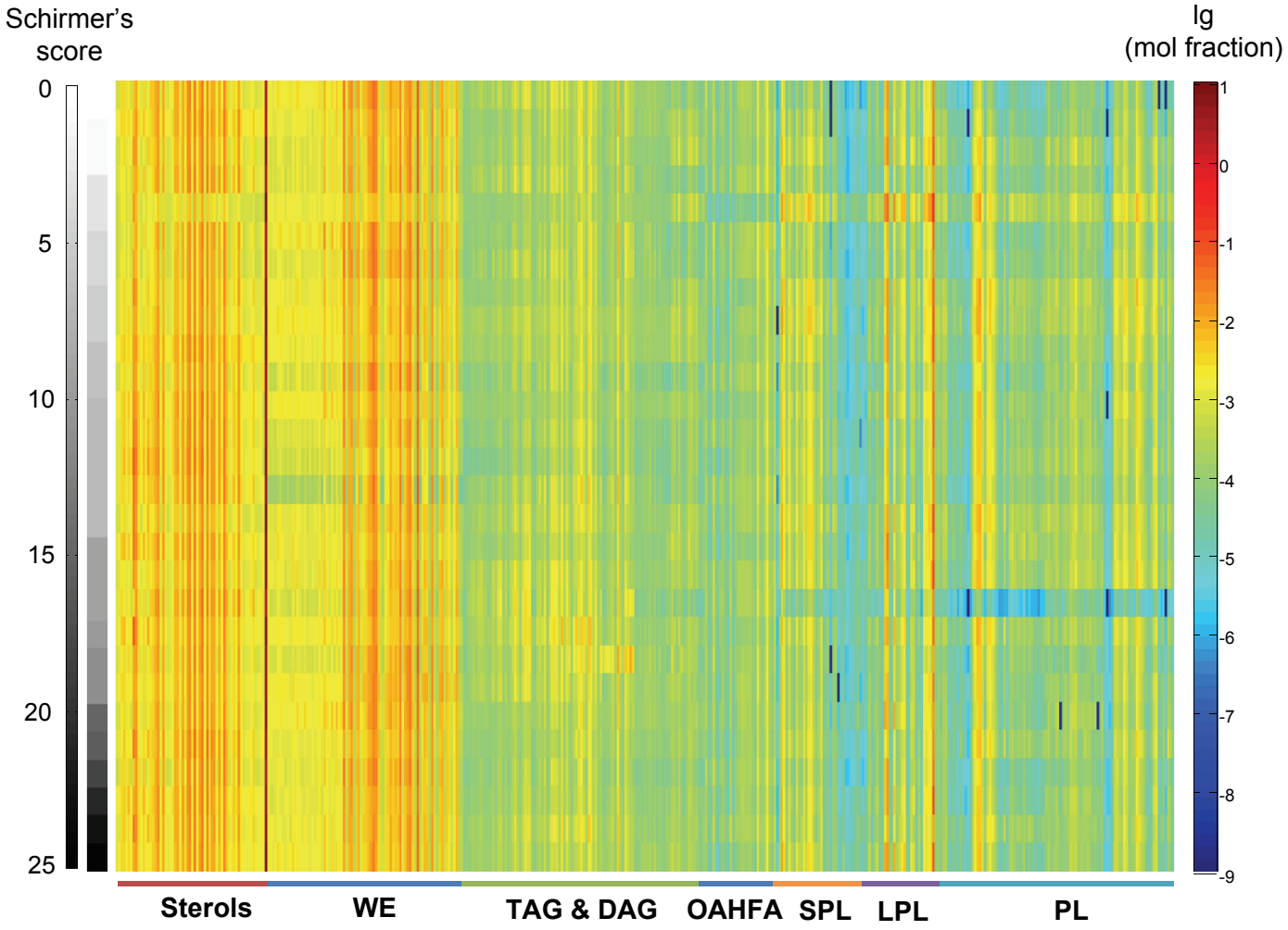


Figure S6, related to Figure 3B. Comparison of DAG profiles between tears and meibum. Lipid profiles were obtained from pooled samples of Schirmer's tears (n=8) and meibum,(n=7) respectively. Values were plotted as $\log_{10}$ of the mol fraction of individual species, and a value of -9 indicates undetectable in the respective samples.

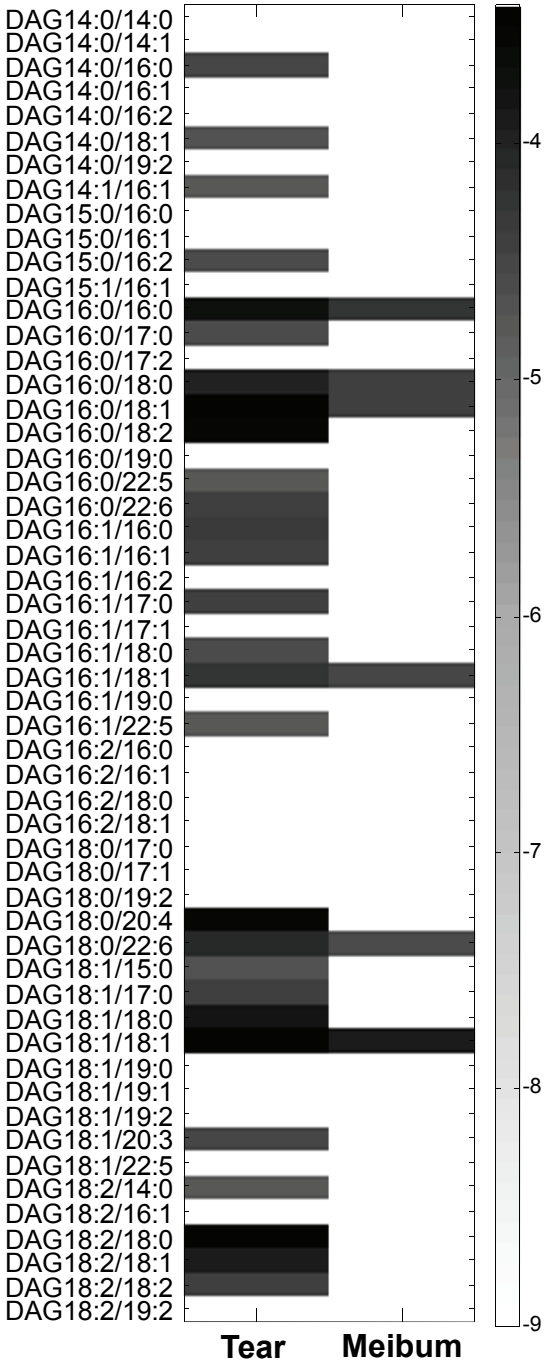


Table S1, related to Figure 1. Validation of Schirmer's strip method of tear collection. Demographic information of four volunteers who provided Schirmer's tear samples for the method validation. Replicate samples were obtained from the same eye at similar timing (1000-1500) on different days to avoid diurnal variations in tear profiles.

Subject No	Sex	Ethnicity	Eye sampled	Schirmer's length (mm)
Subject 1	Male	Chinese	R	22
			R	19
Subject 2	Female	German	R	24
			R	27
Subject 3	Female	Chinese	R	24
			R	19.5
Subject 4	Female	Chinese	R	5.5
			R	7

Table S2, related to Figure 1. Comparison of tear collection techniques. Demographic and clinical information of five volunteers recruited to contribute Schirmer's and capillary-collected tear samples. The volunteers had normal lacrimal function based on the cutoff value given by the report from the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (*i.e.* Schirmer's length > 5.5 mm).

No.	Sex	Age	Ethnicity	Eye	Schirmer's length (mm)	Duration of Capillary Collection (min)	Duration of Capillary collection with 20μL saline flush (secs)
1	M	40	Chinese	R	8	2	20
				L	6		
2	F	25	Chinese	R	24	1*	30
				L	30		
3	F	24	Chinese	R	24	1.5	60
				L	25		
4	F	22	Chinese	R	7	5	60
				L	6		
5	F	32	Chinese	R	19	5	10
				L	14		

* Reflex tearing was observed.

Table S3, related to Figure 1. Summary of the percentages of the major classes of tear lipids detected in the first three segments of the strips relative to the amounts along the entire length of the wetted portions.

	Segment -5-0	Segment 0-5	Segment 5-10	Percent of Total in first 3 segments
TOTAL CE	57.07	33.13	4.09	94.29
TOTAL WE	52.93	30.33	5.21	88.47
TOTAL OAHFA	53.12	32.58	5.78	91.48
TOTAL PC	66.11	8.14	6.57	80.82
TOTAL CER	62.68	9.39	7.32	79.39
TOTAL SM	74.60	8.58	4.67	87.85
TOTAL PS	65.50	8.35	7.87	81.72
TOTAL PI	73.47	5.44	5.47	84.38

Table S4, related to Table 1. The relative abundance of individual classes of lipids in the human tears and meibum. Values were present as percentage molar fraction (normalized to total lipids). The lipid profiles were obtained using pooled samples of Schirmer's tears and meibum collected from dry eye patients.

	Percent (%)	Tear	Meibum
Neutral Lipids	TOTAL CE	44.8175	49.2656
	TOTAL WE	35.2106	43.0449
	TOTAL FREE CHO	5.9397	1.5566
	TOTAL CS	0.1400	0.2778
	TOTAL TAG	2.8387	2.3849
	TOTAL DAG	0.2978	0.0317
	TOTAL OAHFA	2.5170	2.8754
Lyso-glycerophospholipids	TOTAL LPC	0.4937	0.0242
	TOTAL LPS	0.4632	0.0162
	TOTAL LPA	0.0655	0.0012
	TOTAL LPI	0.0453	0.0037
	TOTAL LPE	1.2935	0.0412
Glycerophospholipids	TOTAL PC	2.4687	0.2004
	TOTAL PS	0.1104	0.0104
	TOTAL PA	0.0771	0.0071
	TOTAL PI	0.7165	0.0439
	TOTAL PE	0.6974	0.1341
	TOTAL PG	0.0079	0.0013
	TOTAL LBPA	0.0291	0.0024
Sphingolipids	TOTAL SM	1.4967	0.0594
	TOTAL CER	0.2582	0.0162
	TOTAL GLUCER	0.0032	0.0003
	TOTAL GM3	0.0123	0.0011
	TOTAL S1P	0.0001	0.0000

Table S5, related to Figure S4. Demographic and clinical information of the dry eye cohort used for validation of analytical methods. A total of 28 patients were included from which both Schirmer's tear and meibum samples were analyzed. Entry criteria include TBUT≤5 secs; Schirmer's 1 Test ≤ 5.5mm; OSDI >12.9 ; Baylor score > 0. Patients were considered to suffer from certain aspects of dry eye as long as two of the four criteria aforementioned were fulfilled. The entry criteria were not considered stringent as this cohort was only used for validating the translational feasibility of analytical methods developed.

No.	Sex	Age	Race	Eye	TBuT (secs)	Schirmer's length (mm)	OSDI	Baylor score
018	F	55	Chinese	R	3	3	33.3	3
019	F	50	Chinese	R	2	7	16.7	1
020	F	61	Chinese	L	2	10	0.0	7
021	F	55	Chinese	L	4	25	43.8	5
022	F	77	Chinese	L	1	3	58.3	4
023	M	58	Chinese	L	3	0	16.7	7
024	M	50	Chinese	L	2	5	20.8	2
026	F	49	Chinese	R	3	9	35.4	3
027	F	63	Chinese	R	3	4	12.5	3
028	F	58	Chinese	R	4	11	27.1	10
029	F	56	Chinese	R	15	23	35.4	1
030	F	55	Chinese	R	2	1	12.5	19
031	M	58	Malay	L	1	18	12.5	2
032	F	52	Chinese	L	2	11	25.0	10
034	F	54	Chinese	R	3	7	12.5	3
036	M	63	Malay	R	2	21	45.8	11
038	M	66	Chinese	R	2	9	10.4	9
041	F	60	Chinese	L	3	9	20.8	2
043	F	60	Chinese	R	6	7	20.8	2
044	F	52	Chinese	L	2	7	43.8	9
045	F	48	Chinese	L	1	15	0.0	7
046	F	62	Chinese	L	2	7	58.3	11
047	F	74	Chinese	R	4	16	43.8	20
049	F	72	Chinese	R	1	4	31.3	1
050	F	54	Chinese	L	2	5	39.6	8
052	F	52	Chinese	R	5	6	31.3	3
054	M	59	Chinese	R	4	1	45.8	0
055	F	69	Malay	L	4	6	0.0	1

Table S6 (see attached spreadsheet). MRM transitions for tear lipid species.