Supporting Information

Single-Cell Solid-Phase Microextraction Coupled with Mass Spectrometry for the Detection of Metabolites at Cellular and Subcellular Levels

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Table S1. Chemical composition of the artificial solution. The solvent was ultrapure water. Angiotensin II and somatostatin were peptides; maltohexaose and maltoheptaose were sugars; PC(34:1) was a phospholipid.

Species	Concentration	Exact molecular weight	Representative m/z peaks in mass spectra (Positive mode)
Angiotensin II	20μmol	1045.5345	524 ($M+2H^+$) 1046 ($M+H^+$) 1068 ($M+Na^+$) 1090 ($M-H^++2Na^+$)
Somatostatin	20μmol	1636.7167	820 ($M+2H^+$) 1638 ($M+H^+$)
Maltohexaose	50μmol	990.3275	1013 ($M+Na^+$) 1029 ($M+K^+$)
Maltoheptaose	50μmol	1152.3803	1075 ($M+Na^+$) 1091 ($M+K^+$)
PC(34:1)	80μmol	759.5776	761 ($M+H^+$) 783 ($M+Na^+$) 1520 ($2M+H^+$)

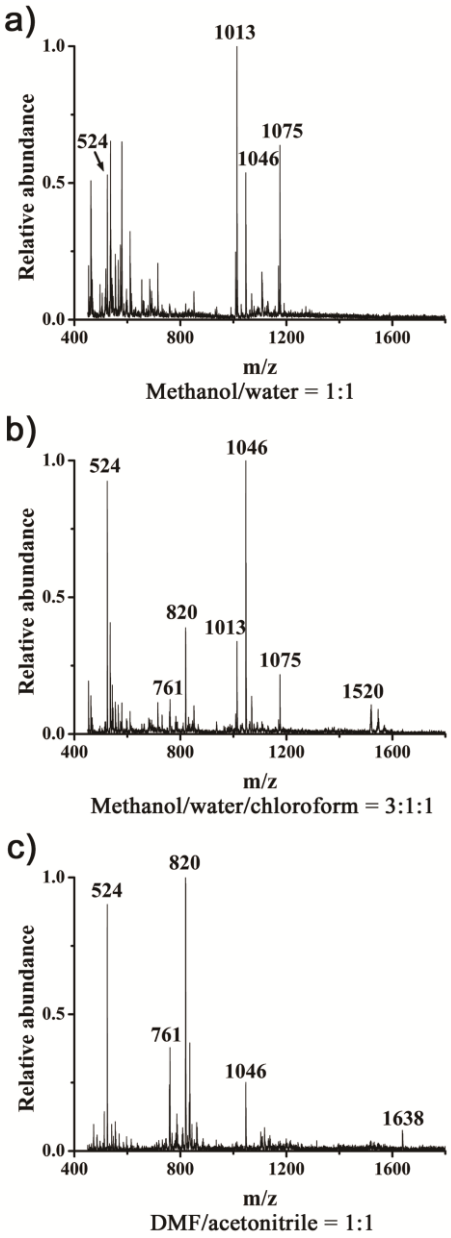

Figure S1. The selectivity of assistant solvents. The artificial solution described in Table S1 was used as the sample. The enrichment time was 5 s. Peak assignment was described in Table S1.

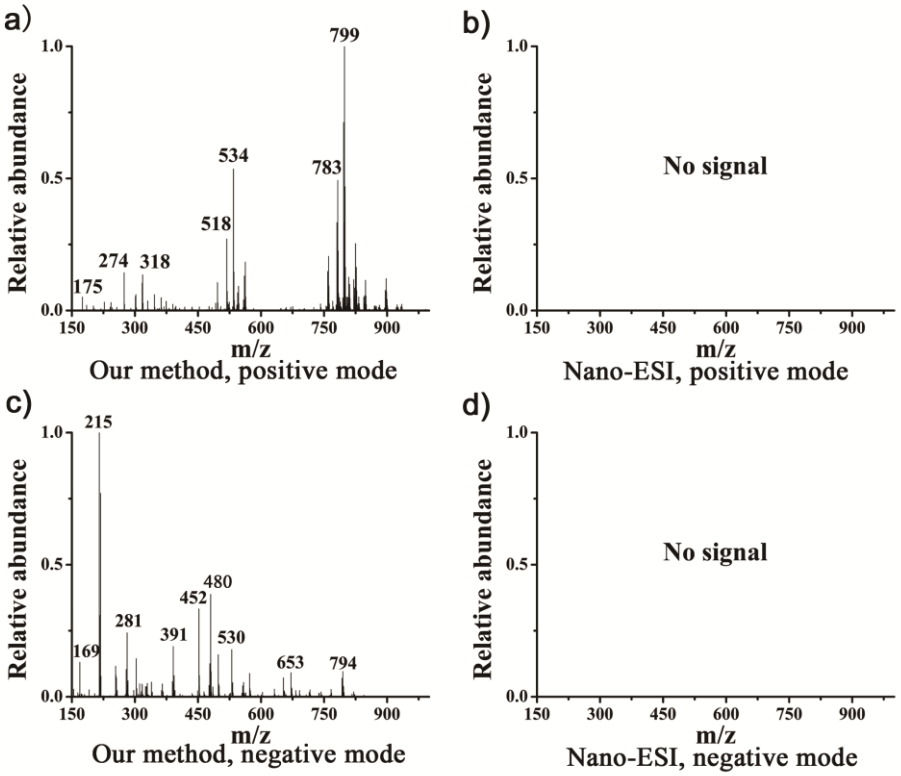

Figure S2. Demonstration of the tolerance of our method to viscosity. Egg yolk was used as the sample. The assistant solvent in a) and c) was methanol/water/chloroform = 3:1:1, and the enrichment time was 5 s.

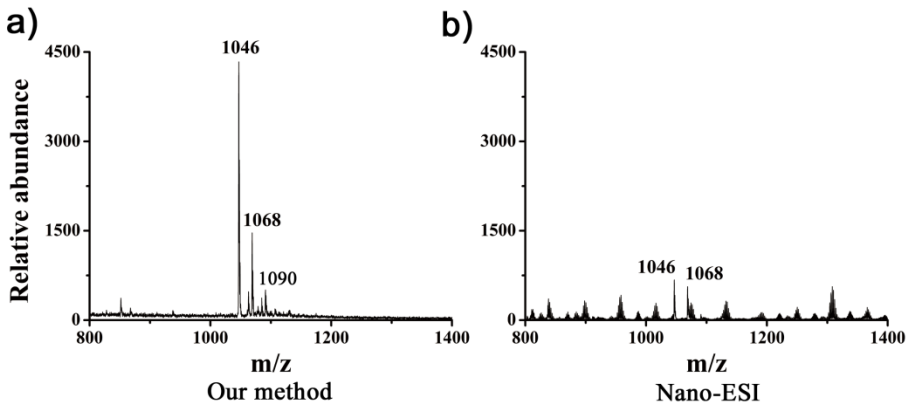

Figure S3. Demonstration of the tolerance of our method to salinity. An aqueous solution with 20 μM angiotensin II and 0.154 M NaCl (physiological saline) was made as the sample. The assistant solvent in our method was methanol/water = 1:1, and the enrichment time was 5 s.

Table S2. Substance identification based on the accurate mass of metabolites.

MS mode	m/z (measured)	m/z (calculated)	Delta m/z (mDa)	Possible Substance	Formula
			Inner epidermal cell		
-	665.2138	665.2141	-0.3	Fructan(DP4)	$C_{24}H_{42}O_{21}(-H^+)$
-	683.2250	683.2247	0.3	Fructan(DP4)	$C_{24}H_{42}O_{21}(+H_2O-H^+)$
-	711.2203	711.2142	6.1	Flavone derivative	$C_{32}H_{40}O_{18}(-H^+)$
-	763.1877	763.1858	-1.9	Flavone derivative	$C_{32}H_{40}O_{19}(+Cl^-)$
-	827.2677	827.2669	0.8	Fructan(DP5)	$C_{30}H_{52}O_{26}(-H^+)$
-	845.2781	845.2775	0.6	Fructan(DP5)	$C_{30}H_{52}O_{26}$ $(+H_2O-H^+)$
-	1007.3312	1007.3303	0.9	Fructan(DP6)	$C_{36}H_{62}O_{31}(+H_2O-H^+)$
-	1169.3836	1169.3831	0.5	Fructan(DP7)	$C_{42}H_{72}O_{36}(+H_2O-H^+)$
-	1331.4359	1331.4359	0	Fructan(DP8)	$C_{48}H_{82}O_{41}(+H_2O-H^+)$
-	1493.4914	1493.4887	2.7	Fructan(DP9)	$C_{54}H_{92}O_{46}(+H_2O-H^+)$
+	689.2136	689.2117	1.9	Fructan(DP4)	$C_{24}H_{42}O_{21}(+Na^+)$
+	705.1865	705.1856	0.9	Fructan(DP4)	$C_{24}H_{42}O_{21}(+K^+)$
+	867.2385	867.2384	0.1	Fructan(DP5)	$C_{30}H_{52}O_{26}(+K^+)$
+	1029.2893	1029.2912	-1.9	Fructan(DP6)	$C_{36}H_{62}O_{31}(+K^+)$
			Outer epidermal cell		
-	661.1172	661.1177	-0.5	Flavone derivative	$C_{27}H_{30}O_{17}$ $(+Cl^-)$
-	759.1654	759.1692	-3.8	Flavone derivative	$C_{36}H_{36}O_{16}$ $(+Cl^-)$
+	665.1147	665.1115	3.2	Flavone derivative	$C_{27}H_{30}O_{17}$ $(+K^+)$
+	758.5731	758.5700	3.1	PE(37:2)	$C_{42}H_{80}NO_8P$ $(+H^+)$
+	782.5730	782.5700	3.0	PE(39:4)	$C_{44}H_{80}NO_8P$ $(+H^+)$
+	796.5292	796.5259	3.3	PC(34:2)	$C_{42}H_{80}NO_8P(+K^+)$
+	820.5288	820.5259	2.9	PC(36:4)	$C_{44}H_{80}NO_8P(+K^+)$

Subcellular detection					
-	463.0872	463.0877	-0.5	Flavone derivative	$C_{21}H_{20}O_{12}(-H^+)$
-	499.0650	499.0643	0.7	Flavone derivative	$C_{21}H_{20}O_{12}(+Cl^-)$
-	625.1403	625.1405	-0.4	Flavone derivative	$C_{27}H_{30}O_{17}(-H^+)$
-	661.1172	661.1172	0	Flavone derivative	$C_{27}H_{30}O_{17}(+Cl^-)$

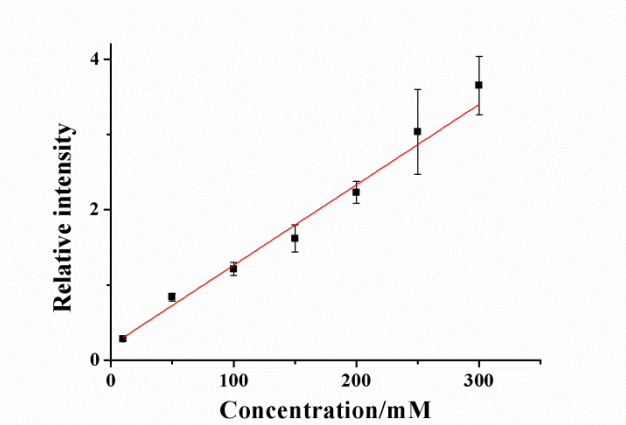

Figure S4. The standard curve for the quantification of sucrose in *A. Cepa* bulb. The enrichment time was 5 s. The assistant solvent was methanol/water = 1:1, with 15mM arabinose in it.

1. Selection of a suitable internal standard.

In general, the internal standard should have similar molecular structure to the analyte. Considering the fact that all the fructans in *A. Cepa* bulb are polymerized by C_6 saccharides, arabinose (C_5 saccharide) were used as the internal standard to avoid interferences from the fructans in A. Cepa bulb.

2. The standard curve.

Sucrose solutions with concentrations of 10, 50, 100, 150, 200, 250, 300 mM were prepared. The assistant solvent was methanol/water = 1:1, with 15mM arabinose in it. The signal intensity of sucrose was compared to arabinose to give a relative intensity ($I_{sucrose}/I_{arabinose}$). The result was shown in Figure R3. The formula of the standard curve was I=0.193+0.0107 C.

3. The quantification of sucrose in *A. Cepa* bulb.

The probe was inserted into the *A. Cepa* bulb to enrich sucrose. The enrichment time was 5 s. Subsequent measurement and calculation gave the final result. The concentration of sucrose in *A.*

Cepa bulb was in the range of 20-50 mM. This result matched well with literatures (10-80 g/g fresh weight)

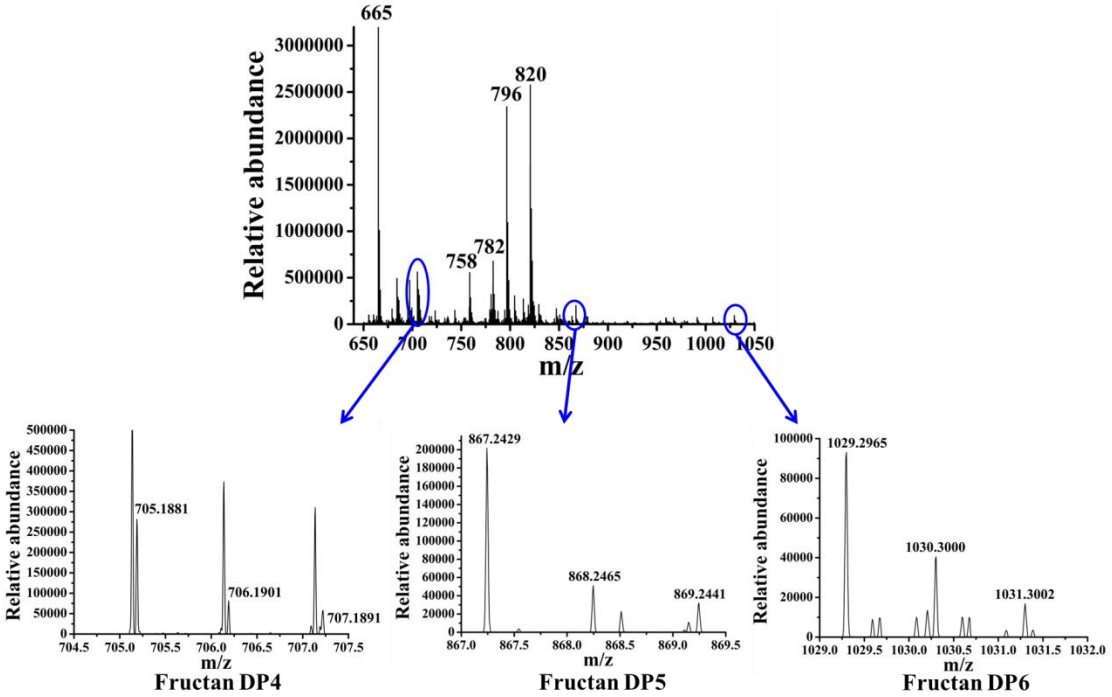

Figure S5. Fructan DP4-DP6 assigned in an outer epidermal cell according to their accurate mass and the isotope peaks.

MS/MS Results of some typical metabolites in *A. Cepa* cells.

1. Fructans.

Fructans are abundant species in *A. Cepa* bulb. Their molecular weight follow the rule: M=18+162n ("n" is the degree of polymerization). In positive mode, fructans are represented by peaks with m/z = 18+162n+23/39(Na^+/K^+) in mass spectra. The MS/MS spectra of fructans are also regular. Figure S6 showed the MS/MS spectra of fructans detected in the inner epidermal cells. It could clearly be seen that fructans with higher DP (Degree of polymerization) values would generate fructan ions with lower DP values. For example, fructan DP2, namely sucrose, generated fructan DP1 ions when fragmented (Figure S6 A). And fructan DP3 generated fructan DP2 and DP1 ions when fragmented (Figure S6 B).

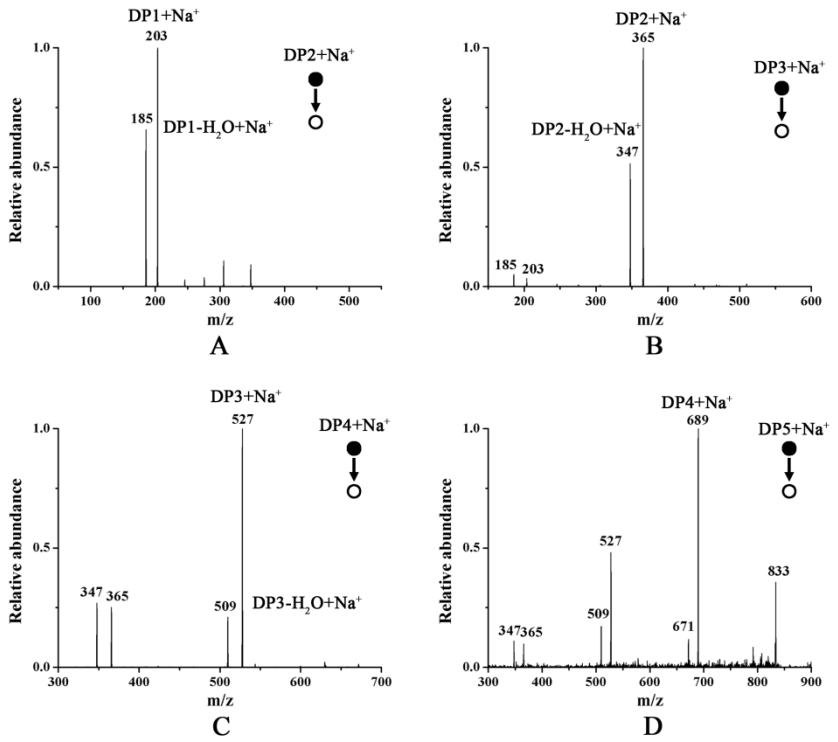

Figure S6. MS/MS spectra of fructans detected in the inner epidermal cells. A) MS/MS spectrum of fructan DP2. B) MS/MS spectrum of fructan DP3. C) MS/MS spectrum of fructan DP4. D) MS/MS spectrum of fructan DP5.

In comparison, the MS/MS spectra of standard saccharide samples (Sucrose and melezitose) were also investigated. The results were shown in Figure S7. Similar fragmentation rules could be figured out compared to those got from the fructans in the inner epidermal cells of *A. Cepa* cells. Taking into considerations of the results of accurate mass measurements and literatures, we came to the conclusion that the peaks with m/z = 18+162n+23/39(Na^+/K^+) in the positive mass spectrum of the inner epidermal cells represented fructans.

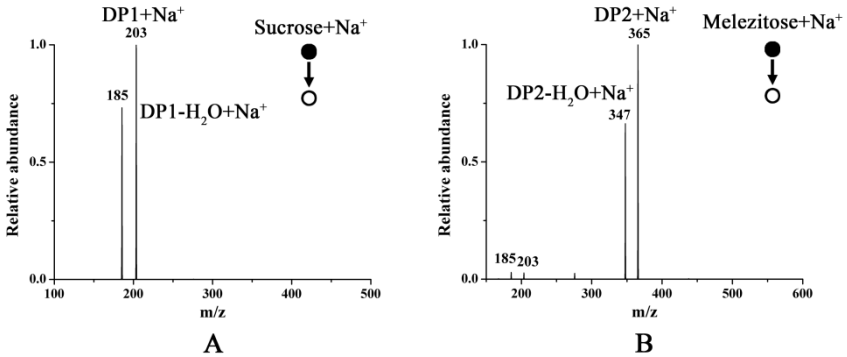

Figure S7. MS/MS spectra of standard saccharide samples. A) MS/MS spectrum of sucrose. B) MS/MS spectrum of melezitose.

2. PCs.

In the positive mass spectrum of the outer epidermal cells, we observed peaks with m/z = 796 and 820. According to accurate mass measurements, these two peaks were supposed to represent PC(34:2) and PC(36:4), respectively. The MS/MS results were shown in Figure S8. Both of the two species lost a fragment with a mass of 59. This was supposed to be caused by the loss of choline residue. Besides, a mass loss of 18 was also observed, which was supposed to be the loss of H_2O.

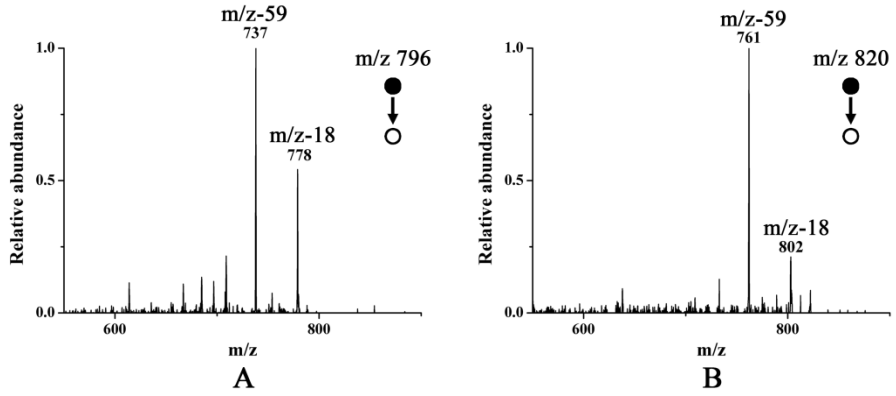

Figure S8. A) MS/MS spectrum of the peak with m/z = 796. B) MS/MS spectrum of the peak with m/z = 820.

In comparison, we investigated the MS/MS spectra of two PC standard samples, namely PC(34:2) and PC(34:1). Similarly, a mass loss of 59 was observed. But no mass loss of 18 was observed. This difference might be caused by the complicated molecular structures of those PC species in *A. Cepa* cells. Taking into considerations of the results of accurate mass measurements and literatures, we came to the conclusion that the peaks with m/z = 796 and 820 in the positive mass spectra of the outer epidermal cells represented PC(34:2) and PC(36:4), respectively.

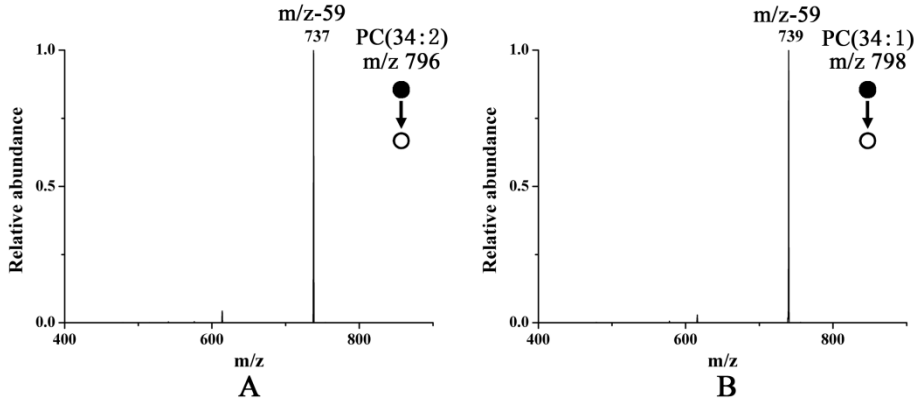

Figure S9. A) MS/MS spectrum of PC(34:2). B) MS/MS spectrum of PC(34:1).

3. Flavonoids.

Flavonoids have been reported to be found in *A. Cepa* bulbs. [Slimestad, R.; Fossen, T.; Vagen, I. M. *J. Agric. Food Chem.* **2007**, 55, 10067-10080] Among those flavonoids found in *A. Cepa* bulbs, many are glycosylated. We successfully identified some glycosylated flavonoids in

the outer epidermal cells. For example, a peak with m/z = 665 was observed in the positive mass spectra of the outer epidermal cells. Further MS/MS experiments were carried out and the results were shown in Figure S10. A typical mass loss of 162 was observed in the MS/MS spectrum. This was quite similar to the MS/MS spectra of the fructans discussed above. Taking into considerations of the results of accurate mass measurements and literatures, we came to the conclusion that the peaks with m/z = 665 in the positive mass spectra of the outer epidermal cells represented a glycosylated flavonoid.

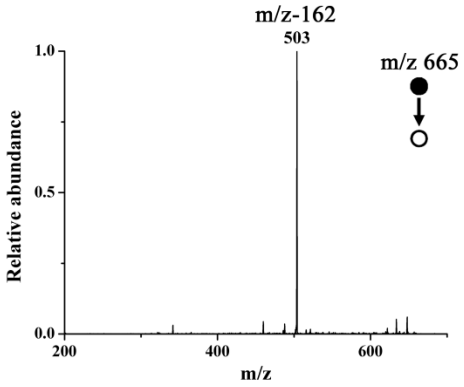

Figure S10. MS/MS spectrum of the peak with m/z = 665 in the positive mass spectra of the outer epidermal cells.