gels

MDPI

Protocol

A LC-QTOF Method for the Determination of PEGDE Residues in Dermal Fillers

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S1. PEGDE qualification method details

The HPLC-QTOF measures were performed on an Agilent 1260 Infinity II HPLC system coupled to an Agilent 6530 Q-TOF mass spectrometer. Chromatographic separation was performed using Agilent InfinityLab Poroshell 120EC C18 column (150 x 3.0 mm, 2.7µm).

Table S1. HPLC-QTOF method details for qualification of PEGDE reference material.

<table>
<tr><th colspan="2">LC</th></tr>
<tr><td>Gradient</td><td>
<table>
<tr><th>Time
(min)</th><th>A*
(%)</th><th>B **
(%)</th><th>Flow
(mL/min)</th></tr>
<tr><td>0.00</td><td>1.0</td><td>99.0</td><td>0.600</td></tr>
<tr><td>1.00</td><td>1.0</td><td>99.0</td><td>0.600</td></tr>
<tr><td>7.00</td><td>80.0</td><td>20.0</td><td>0.600</td></tr>
<tr><td>8.00</td><td>90.0</td><td>10.0</td><td>0.600</td></tr>
<tr><td>9.00</td><td>100.0</td><td>0</td><td>0.600</td></tr>
<tr><td>10.00</td><td>100.0</td><td>0</td><td>0.600</td></tr>
<tr><td>11.00</td><td>1.0</td><td>99.0</td><td>0.600</td></tr>
</table>
* A = 70% ACN+30% H_2O /10 mM Ammonium Formate +0.1% Formic acid
** B = H_2O + 10 mM Ammonium Formate + 0.1% Formic Acid
</td></tr>
<tr><td>Stoptime</td><td>13 minutes</td></tr>
<tr><td>Temperature</td><td>5 °C</td></tr>
<tr><td>Injection Volume</td><td>5 µL</td></tr>
<tr><td>Flow</td><td>0.600 mL/min</td></tr>
<tr><th colspan="2">Q-TOF</th></tr>
<tr><td>Drying gas</td><td>8 L/min</td></tr>
<tr><td>Fragmentor</td><td>150 V</td></tr>
<tr><td>Vcap</td><td>3500 V</td></tr>
<tr><td>Ion polarity</td><td>Positive</td></tr>
</table>

Mass range (m/z)	100-1000
Acquisition Rate	2 spectra/s

S2. PEGDE quantification method details

The HPLC-QTOF measures were performed on an Agilent 1260 Infinity II HPLC system coupled to an Agilent 6530 Q-TOF mass spectrometer. Chromatographic separation was performed using Agilent InfinityLab Poroshell 120EC C18 column (150 x 3.0 mm, 2.7μm).

Before the starting of the measurements, the system tuning was performed with undiluted ESI-L Tune/Calibration mix TOF (G1969-85000) supplied by Agilent Technologies. Ions 121.050873. and 922.009798 were selected as reference ions during the measurements, coming from a reference solution prepared by diluting the G1969-85001 ES-TOF Reference Mass Solution Kit in acetonitrile/water according to Agilent instructions.

Table S2. HPLC-QTOF method details for method validation for PEGDE content in HA hydrogels.

<table>
<tr><th colspan="2">LC</th></tr>
<tr><td>Gradient</td><td>
<table>
<tr><th>Time
(min)</th><th>A*
(%)</th><th>B **
(%)</th><th>Flow
(mL/min)</th></tr>
<tr><td>0.00</td><td>5.0</td><td>95.0</td><td>0.600</td></tr>
<tr><td>1.00</td><td>5.0</td><td>95.0</td><td>0.600</td></tr>
<tr><td>6.00</td><td>65.0</td><td>35.0</td><td>0.600</td></tr>
<tr><td>7.00</td><td>65.0</td><td>35.0</td><td>0.600</td></tr>
<tr><td>8.00</td><td>100.0</td><td>0</td><td>0.600</td></tr>
<tr><td>9.00</td><td>100.0</td><td>0</td><td>0.600</td></tr>
<tr><td>10.00</td><td>5.0</td><td>95.0</td><td>0.600</td></tr>
</table>
* A = 70% ACN+30% H_2O /5 mM Ammonium Formate +0.1% Formic acid
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<tr><td>Ion polarity</td><td>Positive</td></tr>
<tr><td>Mass range (m/z)</td><td>100-1000</td></tr>
</table>

Acquisition Rate	2 spectra/s

S3. PEGDE Certificate of Analysis

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Certificate of Analysis

(Poly(ethylene glycol) diglycidyl ether, Mn=500

CAS No.	72207-80-8	***Batch No.***	B20U06043
Synonyms	α,ω-Diglycidyl poly (ethylene glycol) - 500	***Packing Quantity***	10 g
TESTING ITEM	***SPECIFICATION***	***RESULT***	
Appearance	Colorless to yellowish liquid	Yellowish liquid	
Color	≤ 4	Conforms	
Identity (HNMR) *	Conforms to structure	Conforms	
Substitution (HPLC) *	≥ 93.0%	99.0%	
Average molecular weight *	500 ± 50 Da	534 Da	
Moisture (K.F.) *	≤ 2.0%	0.01%	
Residual solvents *	Dichloromethane ≤ 600 ppm	50 ppm	
	Tetrahydrofuran ≤ 720 ppm	50 ppm	
	Toluene ≤ 890 ppm	50 ppm	
	n-Heptane ≤ 5000 ppm	50 ppm	
Endotoxins *	≤ 0.1 EU/mg	0.1 EU/mg	
Conclusion	The product complies with the given specifications.		
Storage	-20 °C		

QC: Harry Garrett **Approved by:** Zoey Tseng **Date:** June 28, 2020

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Figure S1. Py-GC-MS mass spectra comparison of (a) the peak at RT= 21.75 in the first shot and (b) the peak at RT=21.43 min in the second shot.

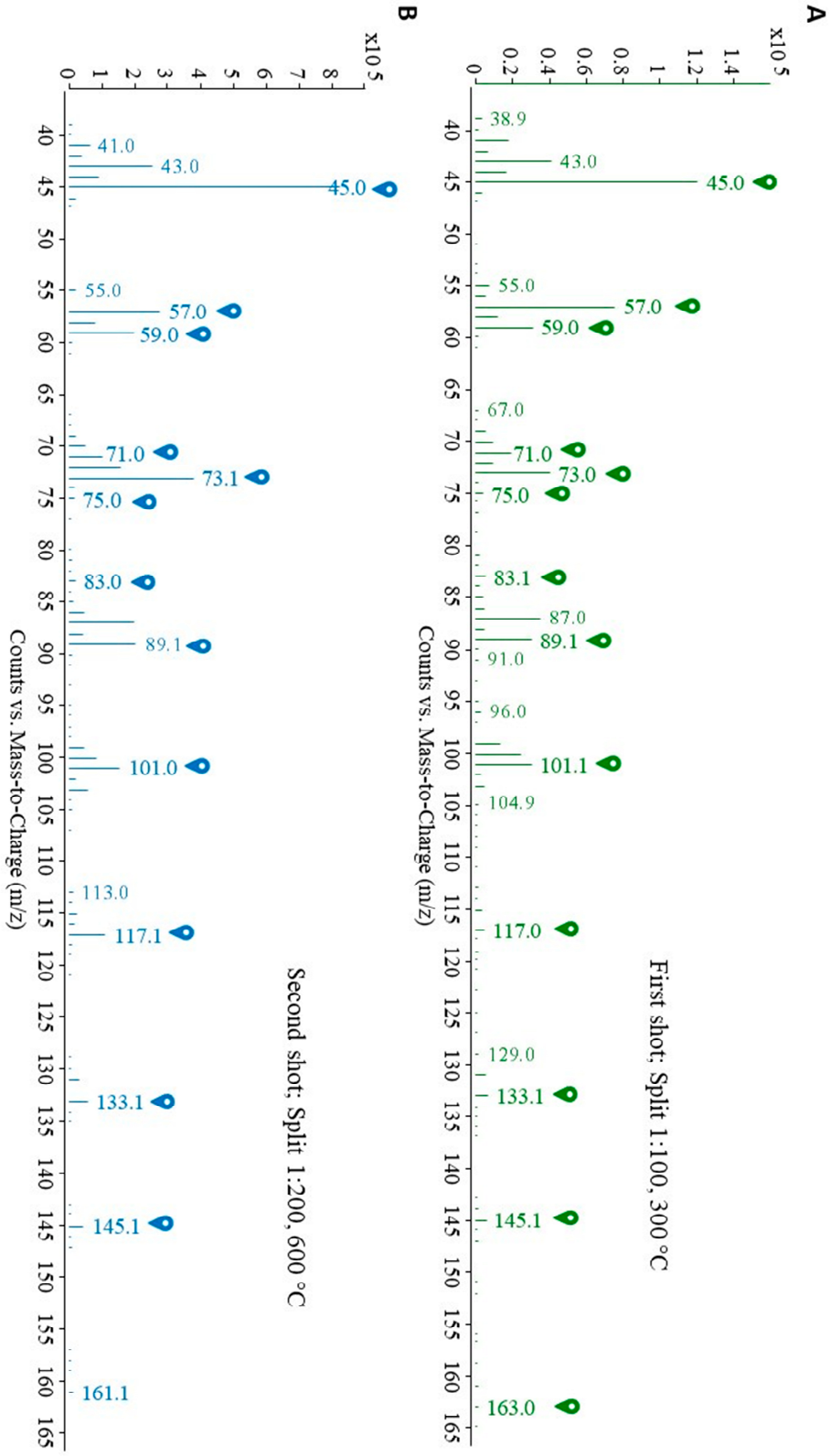

Figure S2. Fragment identification in Py-GC-MS mass spectra of the peak at RT= 21.75 in the first shot.

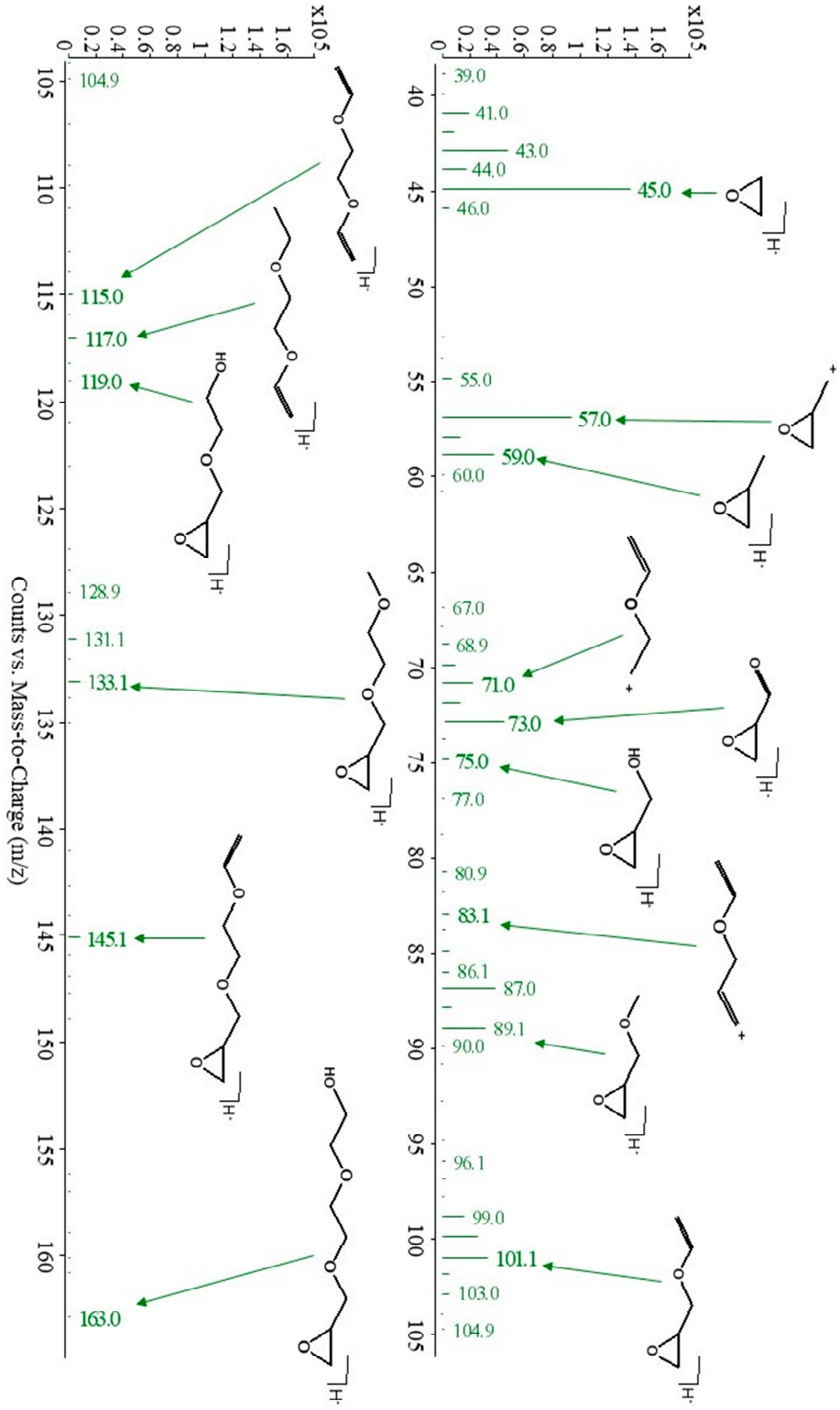

Figure S3. PEGDE fragmentation.