

Isotope-dilution LC-MS/MS analysis of the elastin crosslinkers desmosine and isodesmosine in acute cerebral stroke patients

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Contents

S3: Outline of LC-MS/MS analysis

S4-S5: Figures of fragmentation

S6: MS chromatogram

S7-S8: Optimized LC-MS/MS method

S9-S11: Information of Calibration samples

S12-S15: Information of Plasma samples

S16: Data used for calculations

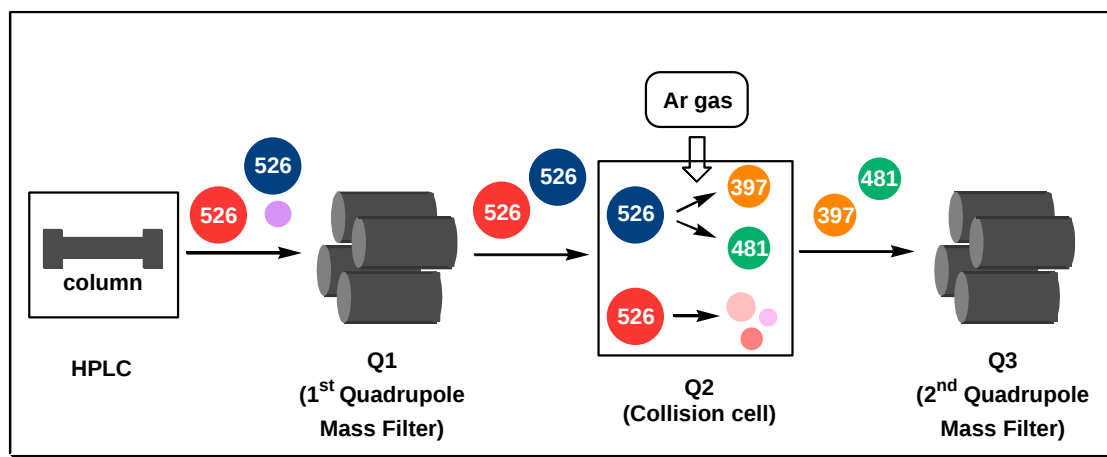


Figure S0. Outline of LC-MS/MS system.

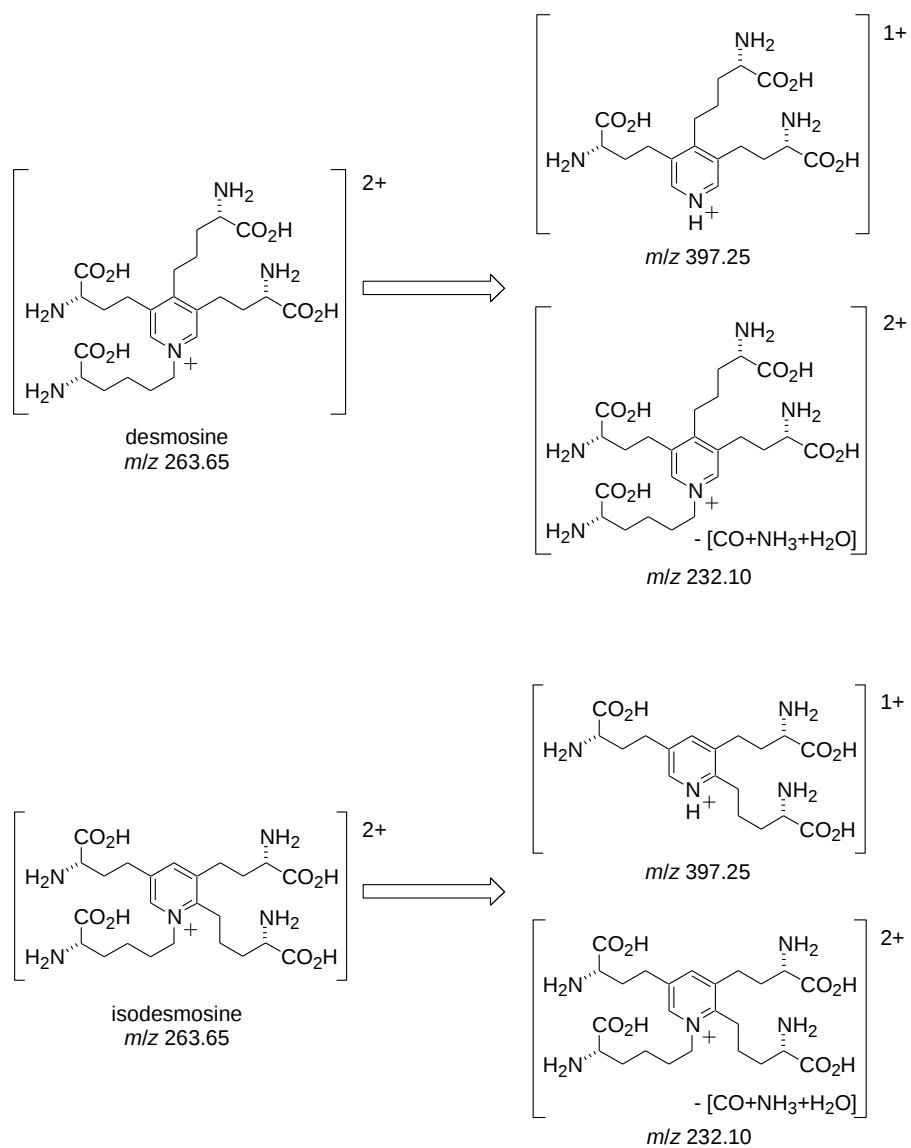


Figure S1. MS/MS fragments of desmosine and isodesmosine.

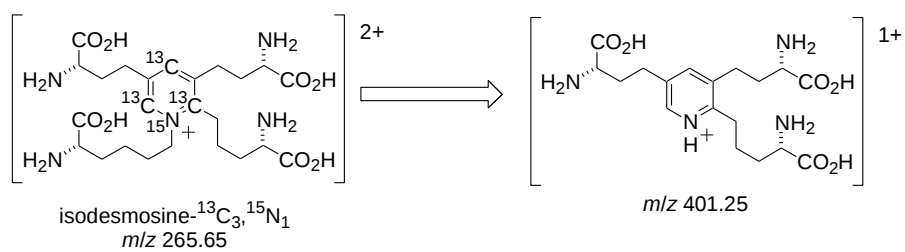


Figure S2. MS/MS fragments of isodesmosine- $^{13}\text{C}_3, ^{15}\text{N}_1$.

Table S1. Optimized LC conditions for desmosine, isodesmosine, and isodesmosine- $^{13}\text{C}_3$, $^{15}\text{N}_1$.

Column:	SUPELCO Discovery HS F5-3 (150mm×2.1mm, Sigma Aldrich)
Mobile phase:	A = 0.1%FA-MeCN, B = 0.1%FA-H ₂ O
	0-4 min: A:B = 90:10
	4-4.5 min: A:B = 90:10-10:90
	4.5-7.5 min: A:B = 10:90-5:95
	7.5-15 min: A:B = 5:95
	15-17 min: A:B = 5:95-90:10
	17-20 min: A:B = 90:10
Flow rate:	0.2 mL/min
Column temp.:	40 °C
Retention time:	12 min

Table S2. Optimized MRM mode MS/MS conditions for desmosine, isodesmosine, and isodesmosine- $^{13}\text{C}_3,^{15}\text{N}_1$.

MRM(+)

Precursor ion (m/z): 263.65 [DES/IDS + H] $^{2+}$

Pause time: 1.0 msec

Dwell time: 400.0 msec

Product ion: Ch1: 232.10, Q1 = -12.0 V, CE = -12.0 V, Q3 = -15.0 V

Ch2: 397.25, Q1 = -11.0 V, CE = -14.0 V, Q3 = -27.0 V

Precursor ion (m/z): 265.65 [IDS (ISTD) + H] $^{2+}$

Pause time: 1.0 msec

Dwell time: 50.0 msec

Product ion: Ch1: 401.25, Q1 = -11.0 V, CE = -14.0 V, Q3 = -27.0 V

*DES: desmosine, IDS: isodesmosine, ISTD: internal standard isodesmosine- $^{13}\text{C}_3,^{15}\text{N}_1$.

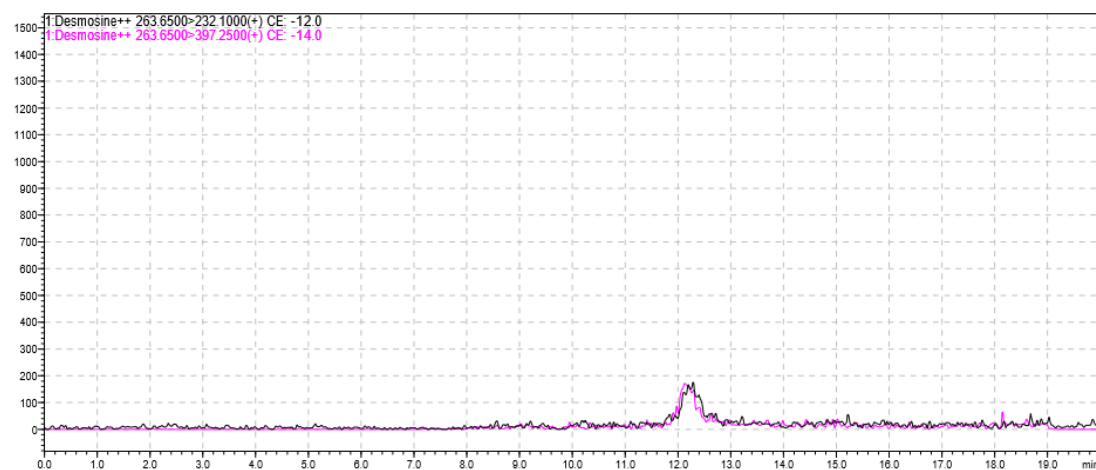
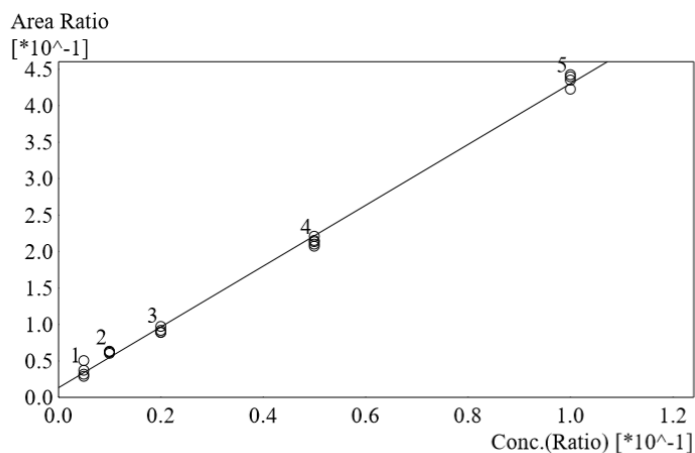


Figure S3. MS chromatogram of 0.005 ppm isodesmosine.

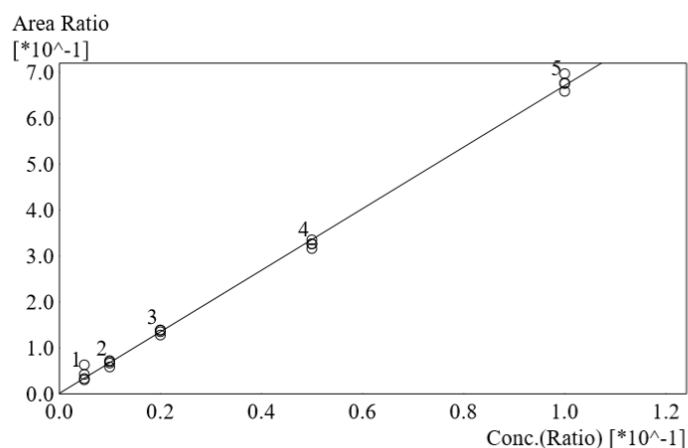
Electronic Supplementary Data

ID# : 2 m/z : 263.6500>232.1000
 Name : Desmosine++
 Quantitative Method : Internal Standard
 Function : $f(x)=4.16503*x+0.0136658$
 Rr1=0.9991964 Rr2=0.9983934
 MeanRF: 5.294163e+000 RF SD: 1.539552e+000 RF %RSD: 29.080179
 FitType : Linear
 ZeroThrough : Not Through



#	Conc.(Ratio)	MeanArea Ratio
1	0.005	0.0370605
2	0.01	0.0619839
3	0.02	0.0917922
4	0.05	0.213312
5	0.1	0.434711

ID# : 3 m/z : 263.6500>397.2500
 Name : Desmosine++
 Quantitative Method : Internal Standard
 Function : $f(x)=6.70020*x+0.00168315$
 Rr1=0.9996481 Rr2=0.9992963
 MeanRF: 7.055989e+000 RF SD: 1.581382e+000 RF %RSD: 22.411908
 FitType : Linear
 ZeroThrough : Not Through

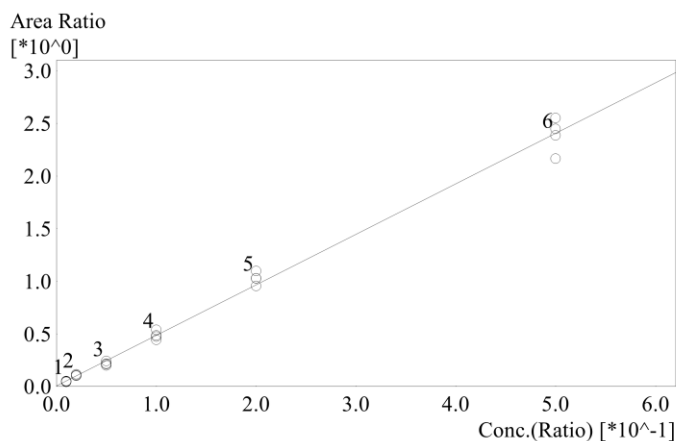


#	Conc.(Ratio)	MeanArea Ratio
1	0.005	0.0424804
2	0.01	0.0673534
3	0.02	0.135170
4	0.05	0.326055
5	0.1	0.676894

Figure S4. Calibration curves of stroke samples (sample S1-S9). Horizontal axis shows area ratio between isodesmosine and isodesmosine- $^{13}\text{C}_3$, $^{15}\text{N}_1$. Vertical axis shows concentration ratio between isodesmosine and isodesmosine- $^{13}\text{C}_3$, $^{15}\text{N}_1$. Top: Calibration curve for fragment 232.10, Bottom: Calibration curve for fragment 397.25.

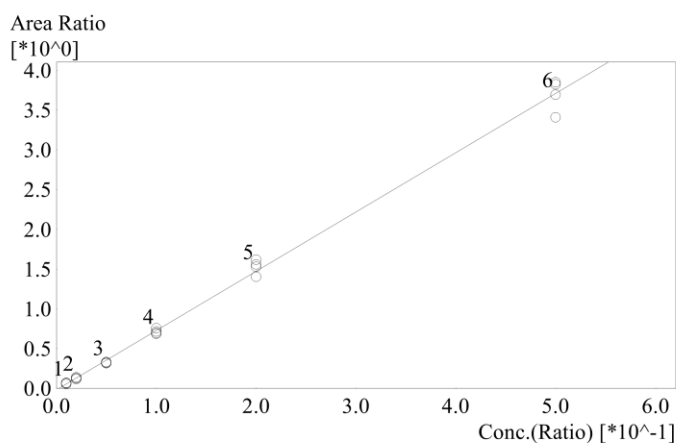
Electronic Supplementary Data

ID# : 2 m/z : 263.6500>232.1000
 Name : Desmosine++
 Quantitative Method : Internal Standard
 Function : $f(x)=4.79754*x+0.00828072$
 $Rr1=0.9993784$ $Rr2=0.9987573$
 MeanRF: 4.871034e+000 RF SD: 4.904789e-001 RF %RSD: 10.069298
 FitType : Linear
 ZeroThrough : Not Through



#	Conc.(Ratio)	MeanArea Ratio
1	0.01	0.0474451
2	0.02	0.107502
3	0.05	0.217298
4	0.1	0.485060
5	0.2	1.02720
6	0.5	2.38701

ID# : 3 m/z : 263.6500>397.2500
 Name : Desmosine++
 Quantitative Method : Internal Standard
 Function : $f(x)=7.45262*x-0.0185897$
 $Rr1=0.9997697$ $Rr2=0.9995395$
 MeanRF: 6.886724e+000 RF SD: 6.786786e-001 RF %RSD: 9.854883
 FitType : Linear
 ZeroThrough : Not Through



#	Conc.(Ratio)	MeanArea Ratio
1	0.01	0.0630300
2	0.02	0.127649
3	0.05	0.323919
4	0.1	0.713847
5	0.2	1.52713
6	0.5	3.69119

Figure S5. Calibration curve of control samples (sample C1-C8). Horizontal axis shows area ratio between isodesmosine and isodesmosine- $^{13}\text{C}_3,^{15}\text{N}_1$. Vertical axis shows concentration ratio between isodesmosine and isodesmosine- $^{13}\text{C}_3,^{15}\text{N}_1$. Top: Calibration curve for fragment 232.10, Bottom: Calibration curve for fragment 397.25.

Table S3. Reproducibility of calibration sample for control sample C1-C8 (fragment: 397.25).

Concentration of isodesmosine/ppm	Area ratio	Mean concentration /ppm	Area ratio SD	Area ratio RSD
0.01 ^{a)}	0.067181	0.012		
	0.055372	0.010	0.0067	0.1054
	0.066559	0.011		
0.02	0.127716	0.020		
	0.138822	0.021	0.0011	0.0878
	0.1164	0.018		
0.05	0.332453	0.047		
	0.321886	0.046	0.0077	0.0239
	0.317407	0.045		
0.1	0.756364	0.104		
	0.697737	0.096	0.0372	0.0521
	0.687451	0.095		
0.2	1.618571	0.220		
	1.558231	0.212	0.1103	0.0722
	1.404665	0.191		
0.5	3.820058	0.515		
	3.406387	0.460	0.2470	0.0669
	3.847154	0.519		

^{a)} Isodesmosine gave sufficient results for quantitation ($S/N > 10$). Since peak picking was slightly difficult under 0.01 ppm of isodesmosine, LOQ was set at 0.01 ppm.

Table S4. Original data of LC-MS/MS area ratio in samples (fragment: 232.10).

Sample name	1st	2nd	3rd	Average	SD	RSD
S1	0.0762255	0.0693671	0.0827004	0.0760977	0.0067	0.0876
S2	0.0640056	0.0675018	0.0657081	0.0657385	0.0017	0.0266
S3	0.0733153	0.061352	0.0685591	0.0677421	0.0060	0.0889
S4	ND	ND	ND	-	-	-
S5	ND	ND	ND	-	-	-
S6	0.060334	0.0702736	0.0516386	0.0607487	0.0093	0.1535
S7	ND	ND	ND	-	-	-
S8	0.062963	0.0621676	0.0609904	0.0620403	0.0010	0.0160
S9	0.0506735	0.0418734	0.0616855	0.0514108	0.0099	0.1931
C1	ND	ND	ND	-	-	-
C2	ND	ND	ND	-	-	-
C3	0.0387784	0.059899	0.0249435	0.0412070	0.0176	0.4272
C4	ND	ND	ND	-	-	-
C5	ND	ND	ND	-	-	-
C6	ND	ND	ND	-	-	-
C7	0.0724753	0.0695055	0.0713483	0.0711097	0.0015	0.0211
C8	ND	ND	ND	-	-	-

Table S5. Original data of LC-MS/MS area ratio in samples (fragment: 397.25)

Sample name	1st	2nd	3rd	Average	SD	RSD
S1	0.0618937	0.0559693	0.0636098	0.0604909	0.0040	0.0663
S2	0.0560117	0.0605046	0.0593762	0.0586308	0.0023	0.0399
S3	0.0475886	0.0567462	0.0666219	0.0569856	0.0095	0.1670
S4	0.0708267	0.0947129	0.0963976	0.0873124	0.0143	0.1638
S5	0.0333272	0.0239662	0.029659	0.0289841	0.0047	0.1627
S6	0.0463502	0.0553055	0.0357188	0.0457915	0.0098	0.2141
S7	0.0337534	0.0293007	0.0272465	0.0301002	0.0033	0.1105
S8	0.0466998	0.0507731	0.0553162	0.0509297	0.0043	0.0846
S9	0.0406519	0.0439314	0.0373193	0.0406342	0.0033	0.0814
C1	ND	ND	ND	-	-	-
C2	ND	ND	ND	-	-	-
C3	0.0487126	0.048881	0.0528261	0.0501399	0.0023	0.0464
C4	ND	ND	ND	-	-	-
C5	ND	ND	ND	-	-	-
C6	ND	ND	ND	-	-	-
C7	ND	ND	ND	-	-	-
C8	ND	ND	ND	-	-	-

Table S6. Concentration of desmosine and isodesmosine in original human plasma.

Sample name	Desmosine/ppm	Isodesmosine/ppm	Sum/ppm
S1	0.00301	0.00258	0.00559
S2	0.00111	0.00389	0.00500
S3	0.00194	0.00311	0.00505
S4	0	0	0
S5	0	0	0
S6	0.00296	0.00137	0.00432
S7	0	0	0
S8	0.00206	0.00250	0.00456
S9	0.00204	0.00163	0.00367
C1	0	0	0
C2	0	0	0
C3	0.00202	0.00297	0.00499
C4	0	0	0
C5	0	0	0
C6	0	0	0
C7	0	0	0
C8	0	0	0

Table S7. The volume of plasma samples before concentration.

Sample name	The volume of plasma sample/ μ L
S1	350
S2	350
S3	350
S4	350
S5	350
S6	350
S7	350
S8	350
S9	350
C1	450
C2	450
C3	450
C4	450
C5	400
C6	400
C7	450
C8	450

Table S8. Constants obtained by synthetic desmosines.

	stroke (sample S1-S9)	control (sample C1-C8)
<i>Cda</i>	3215	6680
<i>Cdb</i>	2121	5268
<i>Cia</i>	2817	2356
<i>Cib</i>	2744	4260