Supplementary Information

Assay Method Development for Tofersen by IP RP LC-HRMS with extracting ion chromatogram processing approach

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List of Supplementary Information

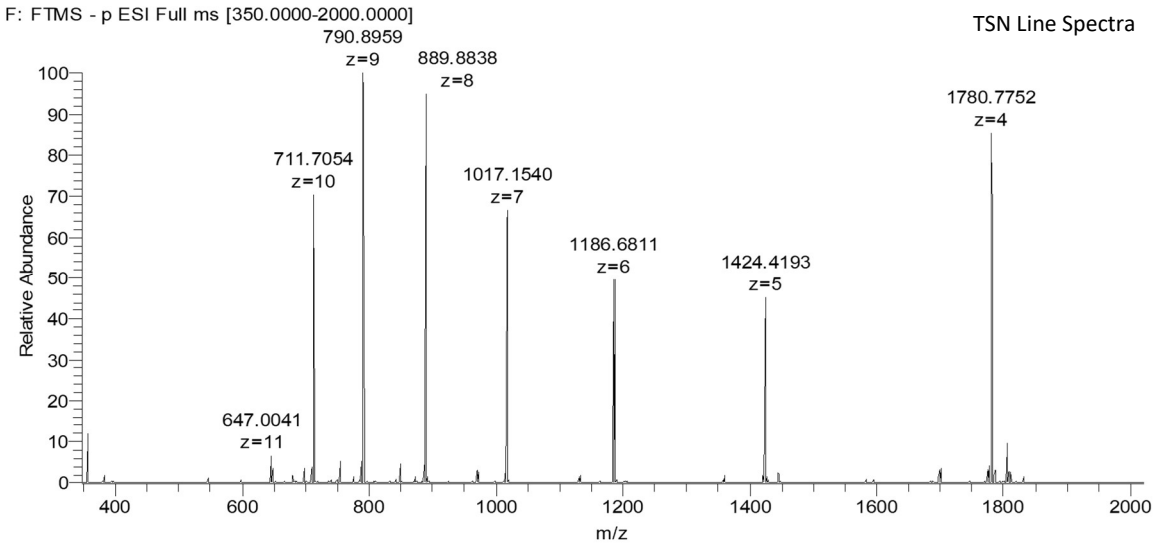

Fig. S1-multiple charged enveloped for molecular mass of TSN within charge (z=4 to z= 11)

The theoretical molecular mass of TSN is 7123.1589 Da. During electrospray ionization, TSN forms a distribution of multiply charged ions that are detected by high-resolution mass spectrometry (HRMS). The resulting mass spectrum showed a series of multiply charged species ranging from 4 to 11, with a mass accuracy error of less than ± 5 ppm.

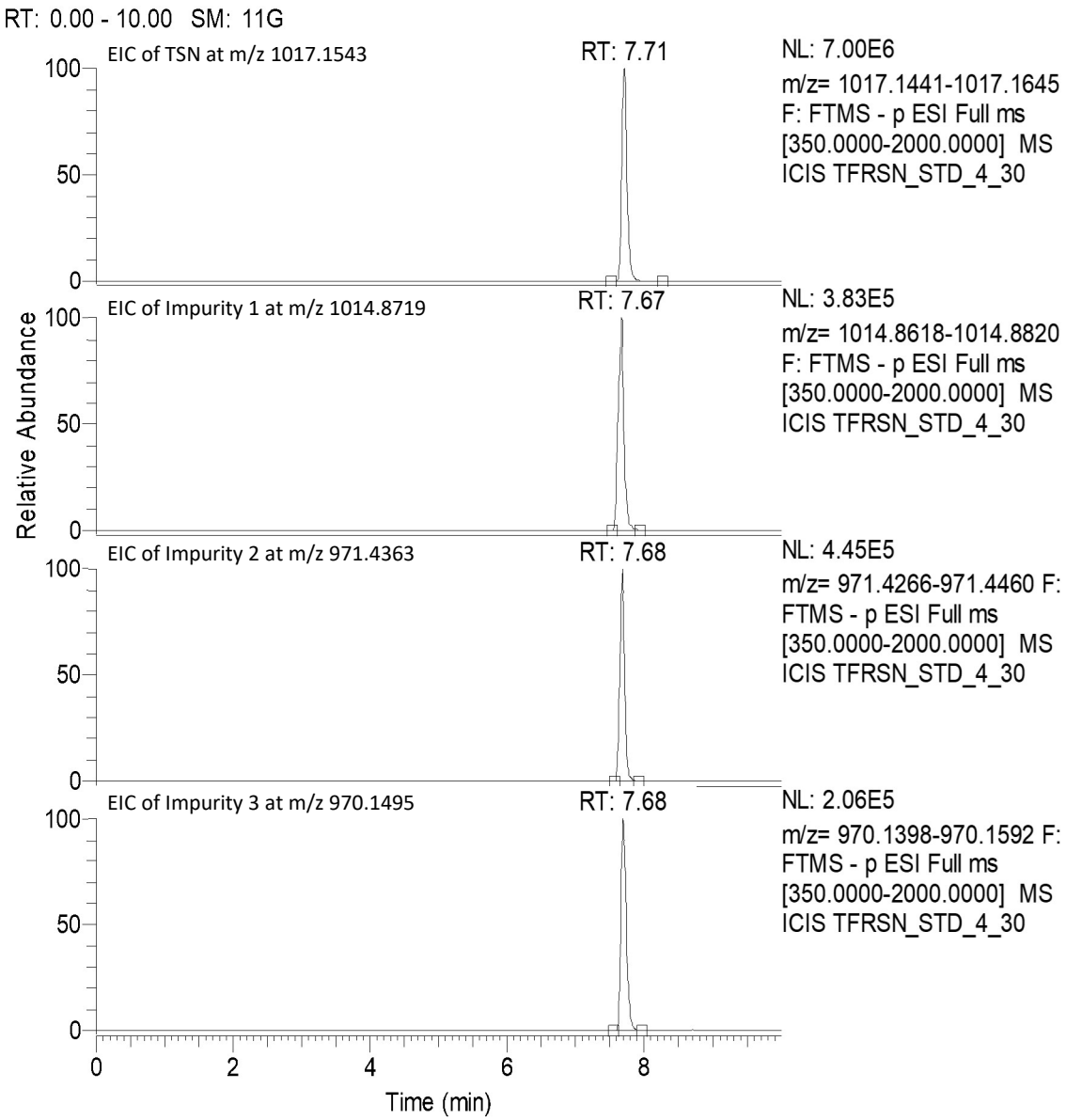

Fig. S2-Extracted ion chromatogram for TSN and it's coeluting impurities.

TSN exhibited an elution time of 7.71 minutes, as determined by extracted ion chromatogram (EIC) at m/z 1017.1543. A negligible difference of less than 0.03 minutes was observed between the retention times of TSN and its co-eluting impurities, precluding their practical separation under the quality control short method.

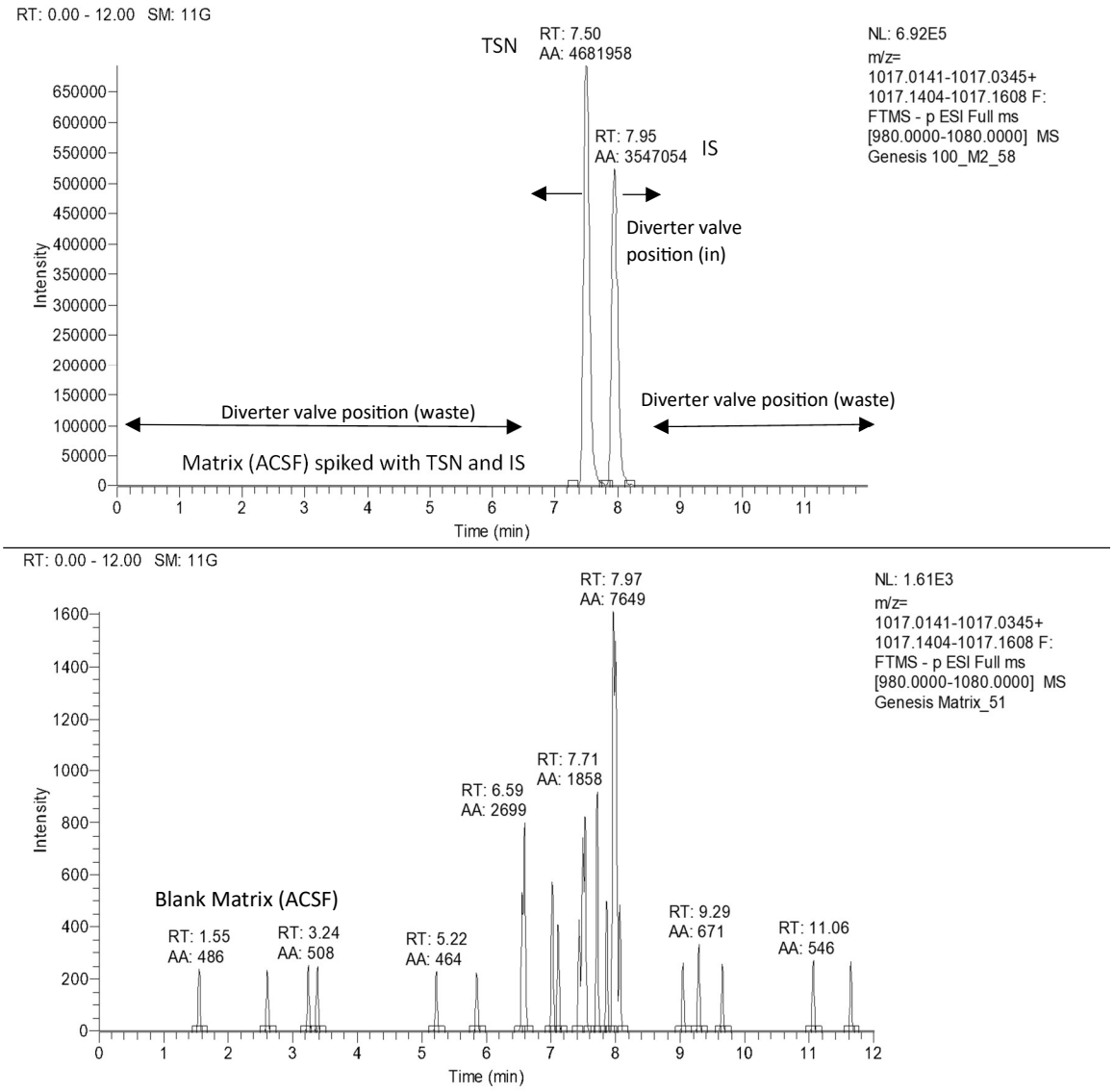

Fig. S3-Specificity of ISN and IS in presence of ACSF considered as formulation matrix

Specificity was evaluated by analysing TSN in the presence of an in-house prepared formulation matrix, which is an artificial cerebrospinal fluid. The matrix was formulated in accordance with the specifications outlined in the USFDA packaging insert for Qalsody. To prevent ionic contamination of the mass spectrometer, the initial 6.5 minutes of chromatographic flow were diverted to waste.

Table S1: Tofersen and co-eluting impurities intact mass Analysis

R.T (min)	m/z	z	Deconvoluted mass (Da)
TSN			
7.71	1779.7725	4	7123.1210
	1423.6167	5	7123.1230
	1186.1777	6	7123.1130
	1016.5815	7	7123.1250
	889.3834	8	7123.1300
	790.4514	9	7123.1330
	711.3047	10	7123.1250
	646.5477	11	7123.1110
Impurity-1			
7.67	1699.5184	4	6802.1048
	1359.4148	5	6802.1130
	1132.6769	6	6802.1082
	970.7222	7	6802.1100
	849.2551	8	6802.1032
	754.7817	9	6802.1055
	679.2038	10	6802.1160
Impurity-2			
7.68	1697.5161	4	6794.0956
	1357.8147	5	6794.1125
	1131.3440	6	6794.1108
	969.5798	7	6794.1132
	848.2566	8	6794.1152
	753.8940	9	6794.1162
	678.4051	10	6794.1290
Impurity-3			
7.68	1775.7807	4	7107.1540
	1420.4238	5	7107.1580
	1183.5191	6	7107.1614
	1014.2996	7	7107.1518
	887.3874	8	7107.1616
	788.6765	9	7107.1587
	709.7081	10	7107.1590
	645.0978	11	7107.1616

Table S2: Robustness of method through LC and MS variable parameters

Method Parameters with Δ change		Mass Accuracy in ppm [(Practical m/z-theoretical mass) *100/theoretical m/z] of TSN at 7th charge			% RSD of Area Ratio
		n=1	n=2	n=3	
LC Flow Rate (mL/min)	0.225	-3.8	-3.6	-3.9	1.8
	0.250	-1.7	-1.4	-1.4	1.7
	0.275	-3.9	-3.8	-3.7	4.9
LC Injection Volume (µL)	4.5	-2.3	-3.0	-3.1	2.2
	5.0	-1.7	1.4	-1.4	1.7
	5.5	-3.6	-3.6	-4.4	4.8
Desolvation Temperature (°C)	300	-3.5	-3.7	-3.8	2.1
	310	-1.7	-1.5	-1.5	1.7
	320	-3.4	-3.7	-3.9	4.6
Gas Flow (Arbitrary Unit)	40	-3.6	-3.6	-4.4	1.7
	44	-3.7	-3.7	-3.8	5.5