pharmaceutics

MDPI

Supplementary Materials: HPLC-Based Analysis of Impurities in Sapropterin Branded and Generic Tablets

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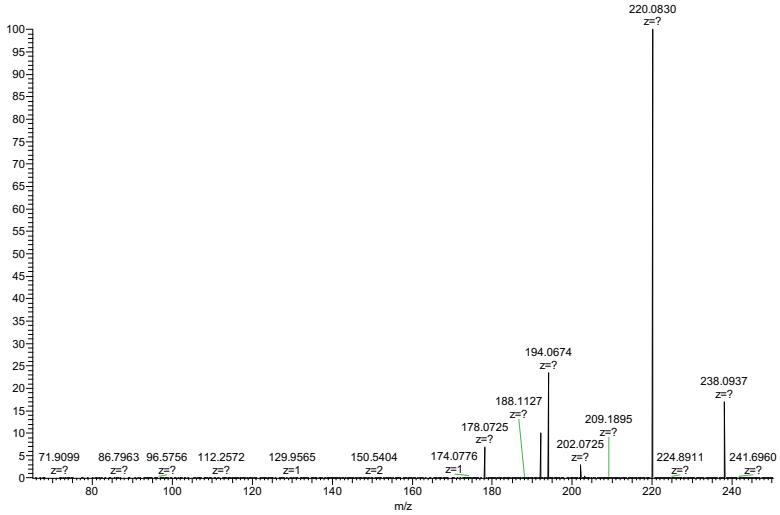
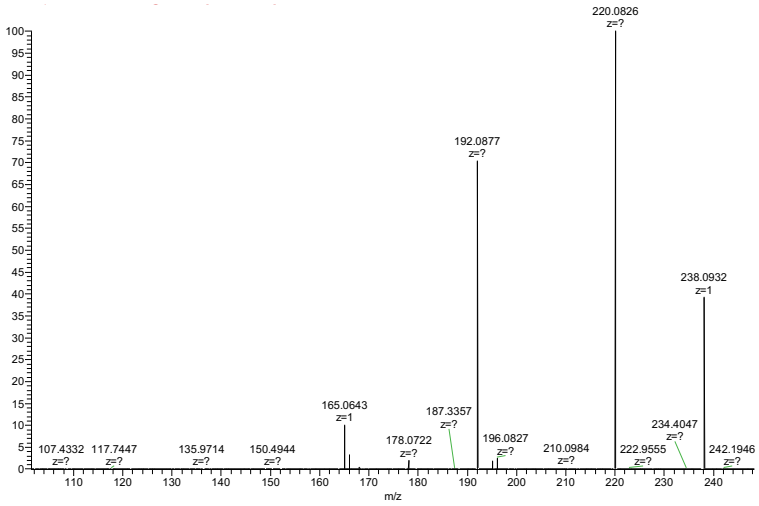
Table S1. Exact masses, molecular formula (MF), Ring Double Bond Equivalents (RDB) and errors (Δppm) measured for $[M+H]^+$ and relevant $[M+Na]^+$ions of each compound contained in Full scan HRMS spectra

Compound	$[M+H]^+$(*m*/*z*) MF, RDB, (Δppm)	$[M+Na]^+$(*m*/*z*) MF, RDB, (Δppm)
BIOPTERIN (1)	238.0936	260.0755
	$C_9H_{12}N_5O_3$, 6.5, (0.480)	$C_9H_{11}N_5O_3Na$, 6.5, (0.344)
SEPIAPTERIN (2)	238.0936	260.0755
	$C_9H_{12}N_5O_3$, 6.5, (0.564)	$C_9H_{11}N_5O_3Na$, 6.5, (0.267)
7,8-DIHYDROBIOPTERIN (3)	240.1093	262.0909
	$C_9H_{14}N_5O_3$, 5.5, (0.725)	$C_9H_{13}N_5O_3Na$, 5.5, (-0.154)
PTERIN (5)	164.0567	186.0387
	$C_6H_6N_5O$, 6.5, (0.571)	$C_6H_5N_5ONa$, 6.5 (0.478)
6*R*-TETRAHYDROBIOLUMAZINE (6)	243.1088	265.0910
6*S*-TETRAHYDROBIOLUMAZINE (7)	$C_9H_{15}N_4O_4$, 4.5, (1.022)	$C_9H_{14}N_4O_4Na$, 4.5, (0.995)
6*S*-TETRAHYDROBIOPTERIN (8)	242.1248	264.1072
	$C_9H_{16}N_5O_3$, 4.5, (1.752)	$C_9H_{15}N_5O_3Na$, 4.5, (1.815)
5,6,7,8-TETRABIOPTERIN (9)	168.0880	190.0700
	$C_6H_{10}N_5O$, 4.5, (0.437)	$C_6H_9N_5ONa$, 4.5, (0.362)

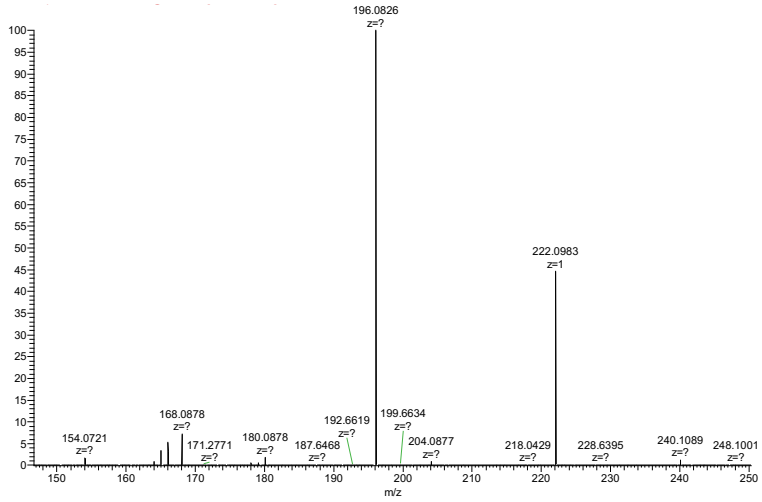
Biopterin (**1**)



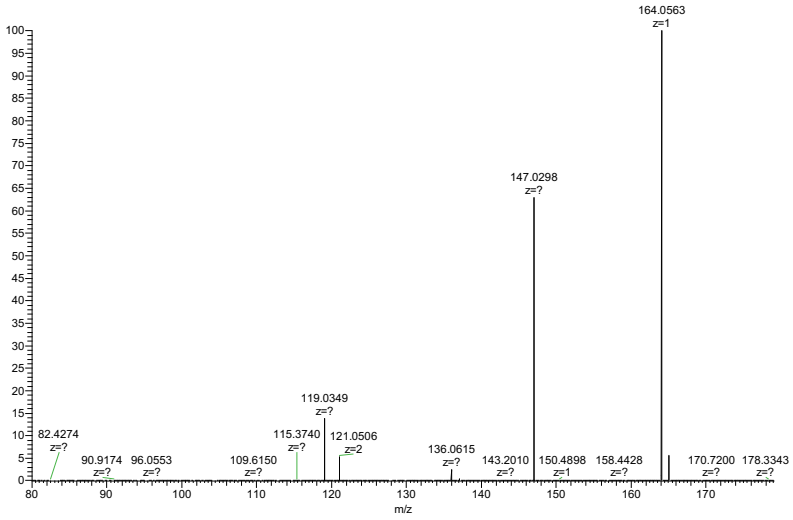
Sepiapterin (**2**)



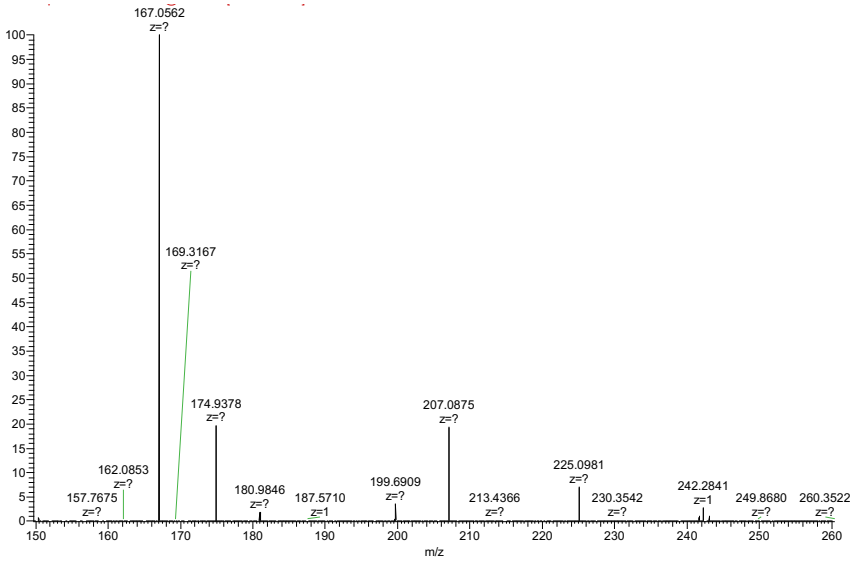
7,8-Dihydrobiopterin (**3**)



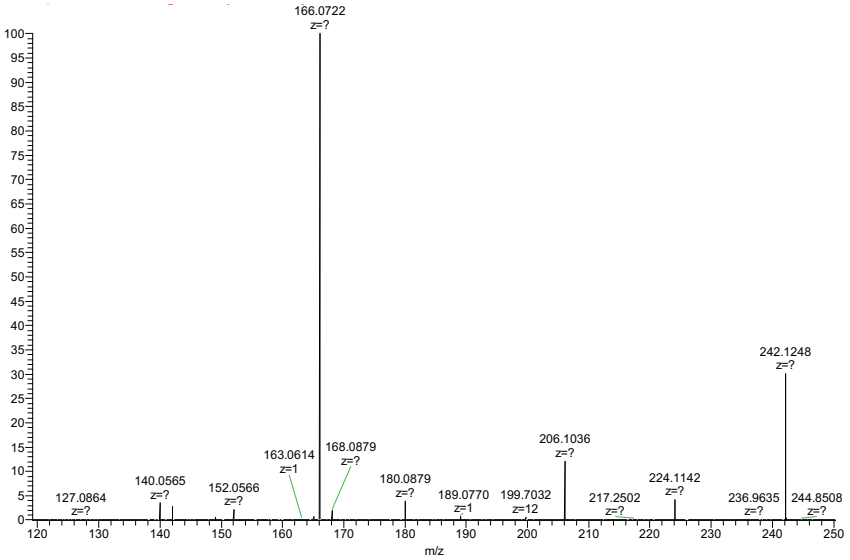
Pterin (**5**)



6*R*-tetrahydrobiolumazine (**6**) and 6*S*-tetrahydrobiolumazine (**7**)



6*S*-tetrahydrobiopterin (**8**)



(5,6,7,8)-Tetrahydropterin (**9**)

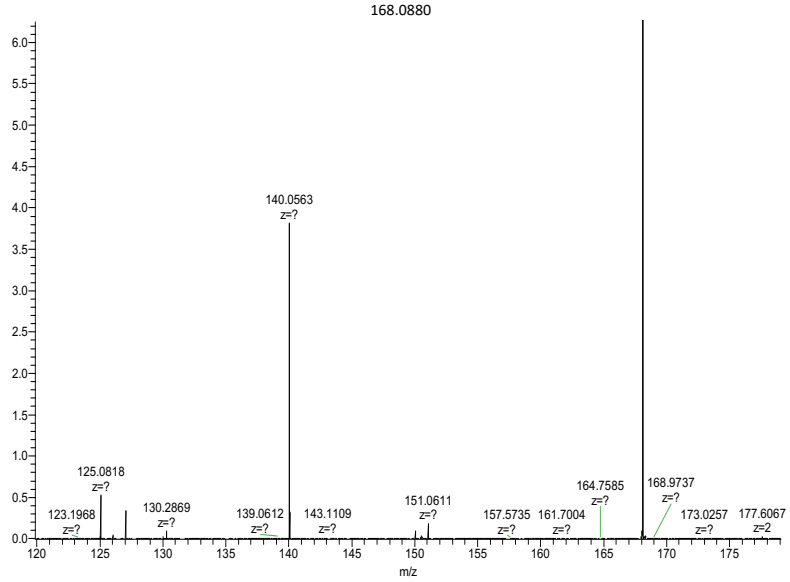


Figure S1. LC-HRMS/MS spectra (CID mode) obtained by selecting the $[M+H]^+$ ion of each impurity/degradation compound as precursor (See Table S1).

Table S2. Exact assignment of the fragment ions contained in the LC-HRMS/MS spectra of compounds **1-3** and **5-9**. Elemental formulae of the mono-isotopic ion peaks (*m*/*z*) are reported with Ring Double Bond Equivalents (RDB) and errors (Δppm). The most intense fragment ion for each compound is in bold.

***m*/*z*, neutral loss** **MF** **RDB, (Δppm)**													
Precursor ions													
1		**2**		**3**		**5**		**6/7**		**8**		**9**	
238.0935 $C_9H_{12}N_5O_3$ 6.5, (0.144)		238.0935 $C_9H_{12}N_5O_3$ 6.5, (0.144)		240.1091 $C_9H_{14}N_5O_3$ 5.5, (-0.066)		**164.0567** **$C_6H_6N_5O$** **6.5, (0.083)**		243.1088 $C_9H_{15}N_4O_4$ 4.5, (0.076)		242.1248 $C_9H_{16}N_5O_3$ 4.5, (0.141)		**168.088** **$C_6H_{10}N_5O$** **4.5, (0.080)**	
Fragment ions													
220.083 **$C_9H_{10}N_5O_2$** **7.5, (0.449)**	-H_2O	**220.0830** **$C_9H_{10}N_5O_2$** **7.5, (0.449)**	-H_2O	222.0986 $C_9H_{12}N_5O_2$ 6.5, (0.220)	-H_2O	147.0300 $C_6H_3N_4O$ 7.5, (-0.934)	-NH_3	225.0982 $C_9H_{13}N_4O_3$ 5.5, (-0.075)	-H_2O	224.114 $C_9H_{14}N_5O_2$ 5.5, (-0.898)	-H_2O	151.0612 $C_6H_7ON_4$ 5.5 (-1.572)	-NH_3
202.0725 $C_9H_8N_5O$ 8.5, (0.809)	-$2H_2O$	192.0881 $C_8H_{10}N_5O$ 6.5, (0.591)	- HCO_2H	**196.0889** **$C_7H_{10}N_5O_2$** **5.5, (-1.026)**	-C_2H_4O	136.0617 $C_5H_6N_5$ 5.5, (-0.528)	-CO	207.0877 $C_9H_{11}N_4O_2$ 6.5, (0.231)	-$2H_2O$	206.1034 $C_9H_{12}N_5O$ 6.5, (-1.148)	-$2H_2O$	140.0565 $C_4H_6N_5O$ 4.5, (-1.331)	-C_2H_4
194.0674 $C_7H_8N_5O_2$ 6.5, (0.768)	-C_2H_4O	178.0725 $C_7H_8N_5O$ 6.5, (0.919)	- $C_2H_4O_2$	180.0878 $C_7H_{10}N_5O$ 5.5, (-1.036)	- $C_2H_4O_2$	121.0507 $C_5H_5N_4$ 5.5, (-1.427)	-CHNO	**167.0563** **$C_6H_7N_4O_2$** **5.5, (-0.311)**	-$C_3H_8O_2$	189.0769 $C_9H_9N_4O$ 7.5, (-0.992)	-NH_7O_2	125.0819 $C_5H_9N_4$ 3.5, (-1.862)	-CHNO
192.0881 $C_8H_{10}N_5O$ 6.5, (0.591)	-HCO_2H	165.0643 $C_6H_7N_5O$ 6.0, (-1.281)	- $C_3H_5O_2$	168.0881 $C_6H_{10}N_5O$ 4.5, (0.675)	- $C_3H_4O_2$	119.0349 $C_5H_3N_4$ 6.5, (-2.711)	-CH_3NO			180.0878 $C_7H_{10}N_5O$ 5.5, (-1.036)	-$C_2H_6O_2$	127.0612 $C_4H_7N_4O$ 3.5, (-1.868)	-C_2H_3N
178.0725 $C_7H_8N_5O$ 6.5, (0.919)	-$C_2H_4O_2$			166.0723 $C_6H_8N_5O$ 5.5, (-0.219)	- $C_3H_6O_2$					168.0878 $C_6H_{10}N_5O$ 4.5, (-1.110)	-$C_3H_6O_2$		
				154.0721 $C_5H_8N_5O$ 4.5, (-1.535)	- $C_4H_6O_2$					**166.0721** **$C_6H_8N_5O$** **5.5, (-1.424)**	-$C_3H_8O_2$		
										165.0644 $C_6H_7N_5O$	-$C_3H_9O_2$		

	152.0564 $C_5H_6N_5O$ 5.5, (-1.883)	$-C_4H_{10}O_2$
	149.0456 $C_6H_5N_4O$ 6.5, (-1.257)	$-C_3H_{11}NO_2$
	142.0721 $C_4H_8N_5O$ 3.5, (-1.664)	$-C_5H_8O_2$
	140.0564 $C_4H_6N_5O$ 4.5, (-2.045)	$-C_5H_{10}O_2$

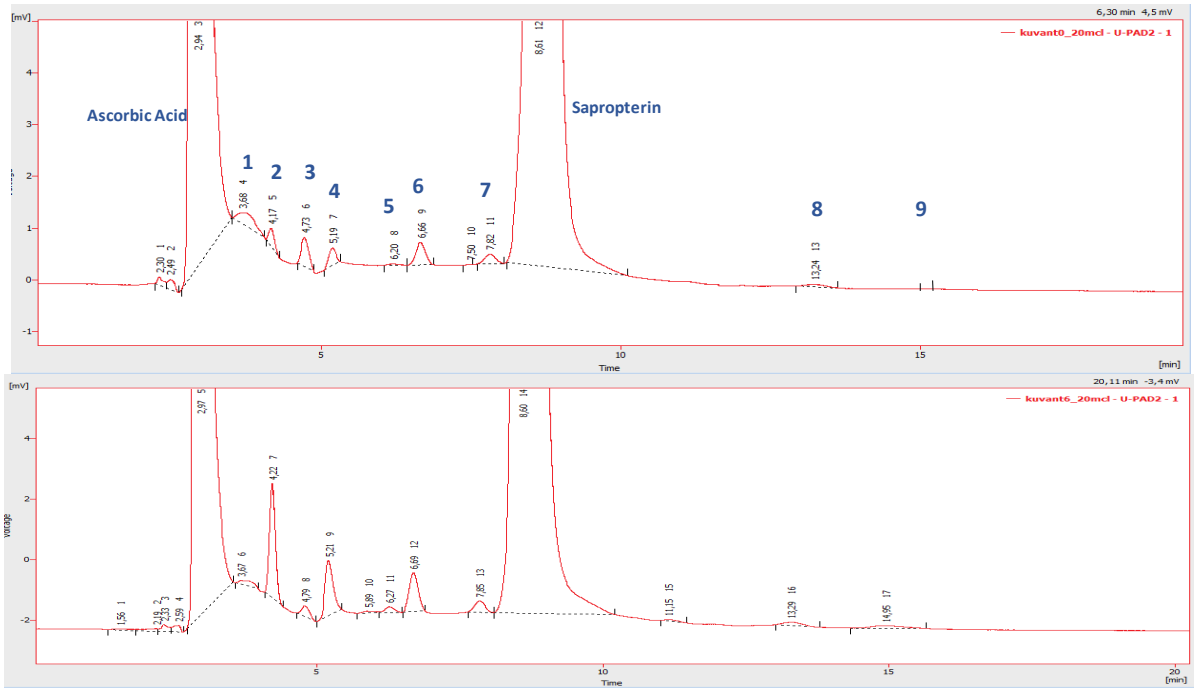


Figure S2. HPLC profile of Kuvan® tablet at T=0 (top) and T = 6 (bottom).

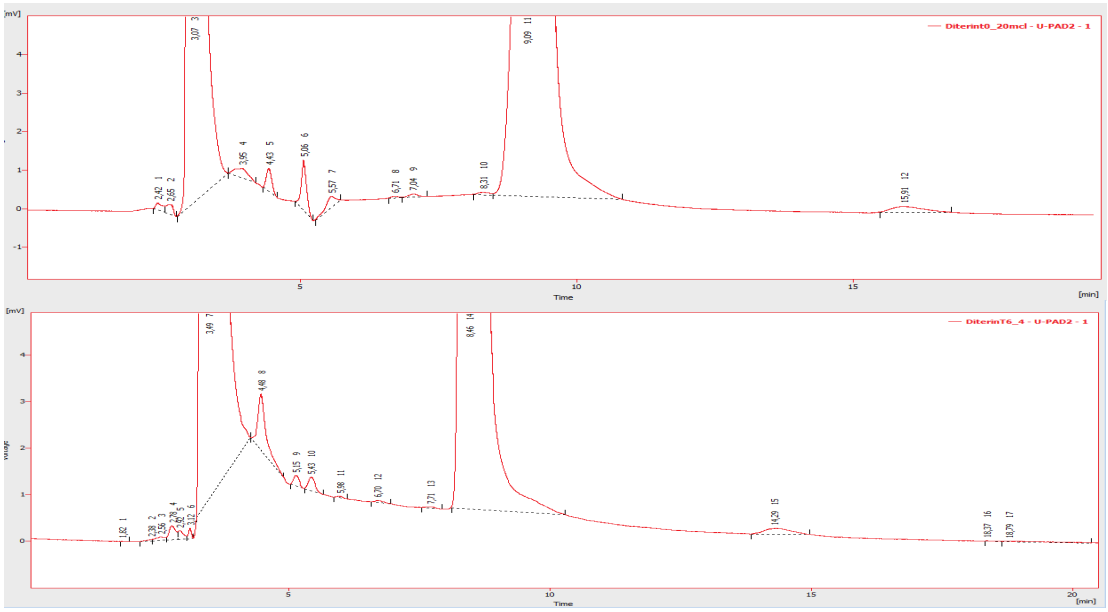


Figure S3. HPLC profile of Diterin tablet at T=0 (top) and T = 6 (bottom).