

Supplementary Material

Table S1. Nominal concentrations of calibration curves and quality control (QC) samples. Concentrations are expressed in mg/L, except for TGB (ng/mL). Compounds are listed in order of retention time apart from CNB. CAL0 = calibrator 0, CAL1 = calibrator 1, CAL2 = calibrator 2, CAL3 = calibrator 3, CAL4 = calibrator 4, CAL5 = calibrator 5, CAL6 = calibrator 6, LLOQ = lower limit of quantification, LQC = lower QC, MQC = medium QC, HQC = high QC, CNB = cenobamate, PGB = pregabalin, GBP = gabapentin, LEV = levetiracetam, ETS = ethosuximide, LTG = lamotrigine, PRM = primidone, LCS = lacosamide, ZNS = zonisamide, RUF = rufinamide, MHD = 10-monohydroxy carbamazepine, BRV = brivaracetam, CBZ-EP = carbamazepine-epoxide, TPM = topiramate, TGB = tiagabine, PMP = perampanel, STP = stiripentol.

Compound	CAL0	LLOQ	CAL1	CAL2	CAL3	CAL4	CAL5	CAL6	LQC	MQC	HQC
CNB	0.00	0.80	2.40	5.00	10.00	20.00	40.00	80.00	3.00	30.00	60.00
PGB	0.00	0.01	0.04	0.08	0.16	0.33	0.65	1.30	0.04	0.04	0.08
GBP	0.00	0.19	0.56	1.17	2.34	4.68	9.35	18.70	0.61	6.10	12.20
LEV	0.00	0.5	1.50	3.13	6.26	12.53	25.05	50.10	1.53	15.30	30.60
ETS	0.00	1.10	3.29	6.84	13.69	27.38	54.75	109.50	3.41	34.05	68.10
LTG	0.00	0.20	0.59	1.23	2.45	4.90	9.80	19.60	0.61	6.10	12.20
PRM	0.00	0.25	0.76	1.58	3.16	6.33	12.65	25.30	0.82	8.20	16.40
LCS	0.00	0.19	0.58	1.21	2.43	4.85	9.70	19.40	0.61	6.10	12.20
ZNS	0.00	0.38	1.15	2.40	4.80	9.60	19.20	38.40	1.20	12.00	24.00
RUF	0.00	0.46	1.38	2.88	5.76	11.53	23.05	46.10	1.45	14.50	29.00
MHD	0.00	0.51	1.52	3.18	6.35	12.70	25.40	50.80	1.58	15.80	31.60
BRV	0.00	0.06	0.19	0.39	7.90	1.58	3.15	6.30	0.22	2.20	4.40
CBZ-EP	0.00	0.08	0.23	0.48	0.96	1.93	3.85	7.70	0.25	2.45	4.90
TPM	0.00	0.28	0.83	1.73	3.46	6.93	13.85	27.70	0.90	9.00	18.00
TGB	0.00	0.40	1.20	2.50	5.00	10.00	20.10	40.10	1.30	13.00	26.00
PMP	0.00	0.03	0.08	0.16	0.33	0.65	1.30	2.60	0.08	0.75	1.50
STP	0.00	0.28	0.85	1.76	3.53	7.05	14.10	28.20	0.87	8.65	17.30

Table S2. Intra-day precision and accuracy of the quantification method in human plasma for the detection of the other detected ASMs. LLOQ = lower limit of quantification, LQC = lower QC, MQC = medium QC, HQC = high QC, CNB = cenobamate, PGB = pregabalin, GBP = gabapentin, LEV = levetiracetam, ETS = ethosuximide, LTG = lamotrigine, PRM = primidone, LCS = lacosamide, ZNS = zonisamide, RUF = rufinamide, MHD = 10-monohydroxy carbamazepine, BRV = brivaracetam, CBZ-EP = carbamazepine-epoxide, TPM = topiramate, TGB = tiagabine, PMP = perampanel, STP = stiripentol.

		PGB					GBP					LEV				
		Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
LLOQ	Bias%	10.0	18.0	-10.0	2.0	10.0	0.7	5.7	-18.0	-16.8	-9.1	4.3	2.7	14.4	-16.6	-4.8
	CV%	6.4	9.3	11.1	8.2	9.1	3.7	3.7	8.6	4.2	3.4	1.3	7.4	6.4	6.6	3.9
LQC	Bias%	-11.0	-6.0	-2.5	-3.0	1.5	-3.4	-7.9	-13.7	8.3	12.3	11.2	2.4	4.3	11.8	14.9
	CV%	7.6	9.7	10.4	5.6	5.9	2.8	3.5	9.0	5.0	3.8	5.9	7.8	4.9	7.6	2.5
MQC	Bias%	2.1	6.8	11.7	-0.1	-1.3	-7.3	-4.5	1.3	-1.9	-5.5	1.1	10.5	10.6	2.7	1.1
	CV%	5.3	3.1	8.2	5.2	4.1	4.6	3.5	5.1	3.1	2.9	2.2	3.5	3.7	2.8	4.7
HQC	Bias%	0.6	4.4	8.5	1.5	0.2	-7.3	-7.8	-1.3	-6.2	-9.9	2.9	10.0	8.8	-1.9	-4.0
	CV%	3.1	2.0	2.4	1.3	3.4	3.2	2.6	3.9	2.1	2.8	4.5	3.7	6.2	4.4	2.6
		ETS					LTG					PRM				
		Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
LLOQ	Bias%	1.4	10.6	-10.7	-11.2	-10.2	8.2	2.6	-18.3	-15.1	-3.1	6.8	-6.1	-13.0	-12.1	11.5
	CV%	2.7	12.3	8.4	6.4	10.3	8.2	11.3	4.7	7.6	2.6	11.6	12.9	11.3	4.3	3.1
LQC	Bias%	1.6	1.2	4.3	3.6	5.2	-1.3	-5.2	5.3	13.1	9.7	-2.6	-5.9	5.0	8.1	11.1
	CV%	4.6	3.3	9.0	6.5	6.7	4.5	2.2	4.5	6.1	5.6	10.3	5.0	7.6	6.4	11.5
MQC	Bias%	-5.6	1.7	-1.0	-8.2	0.2	-5.2	2.3	2.8	3.0	-4.6	-8.0	-9.3	-2.9	-9.1	-3.3
	CV%	7.2	4.2	4.7	6.2	3.0	2.4	5.4	9.4	4.5	3.6	4.8	6.9	9.0	4.6	4.5
HQC	Bias%	-3.9	-2.0	1.4	-9.7	-9.3	-5.9	-3.7	0.3	-1.2	-5.6	-7.4	-7.5	-8.2	-9.3	-2.3
	CV%	2.6	3.3	8.0	3.2	3.5	2.4	2.9	5.0	4.4	3.0	2.9	3.7	4.1	8.0	5.5
		LCS					ZNS					RUF				
		Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
LLOQ	Bias%	13.7	14.2	-6.3	-9.6	-16.8	-5.8	5.2	-6.5	-18.3	-18.7	10.8	10.5	-4.1	-14.8	4.8
	CV%	8.1	6.2	8.0	4.6	6.8	4.2	4.0	6.3	7.2	2.2	8.8	3.2	4.4	5.5	2.3
LQC	Bias%	-1.7	-6.2	1.2	9.4	7.8	-4.3	-6.0	3.9	4.9	10.3	12.6	4.8	7.8	13.3	11.8
	CV%	6.4	2.9	6.9	8.0	3.1	0.8	3.7	5.3	5.3	6.6	7.3	4.0	6.7	7.5	3.0
MQC	Bias%	-7.0	-1.3	-4.3	-2.9	1.9	-4.0	-3.7	-0.8	-2.5	2.7	5.4	4.6	2.1	-0.3	0.6
	CV%	4.5	1.6	7.0	2.7	5.3	4.6	5.0	6.7	4.9	3.2	4.8	1.9	5.9	5.0	6.9
HQC	Bias%	-3.1	-1.5	-6.3	-4.3	-2.5	-3.0	-2.2	-2.4	-5.2	-1.9	9.7	3.4	0.1	-5.5	-3.7
	CV%	1.5	2.7	5.6	3.2	5.0	5.7	3.2	3.0	6.0	3.7	4.0	3.4	7.3	5.3	3.7

		MHD					BRV					CBZ-EP				
		Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
LLOQ	Bias%	-2.9	-2.5	-4.4	-1.3	-9.6	15.4	18.1	-1.0	-10.2	-19.3	-10.0	-7.0	-0.5	-12.5	-9.5
	CV%	1.4	10.7	6.1	3.7	4.9	14.6	9.7	9.2	11.1	7.9	4.6	11.7	6.5	5.9	8.1
LQC	Bias%	-4.4	-14.1	1.8	11.9	5.5	1.0	-13.3	-4.8	-0.2	-3.3	-9.8	-10.2	4.3	2.9	6.7
	CV%	5.9	6.5	4.0	10.8	2.8	4.3	6.4	10.6	3.1	5.5	9.0	7.7	7.7	7.8	5.5
MQC	Bias%	-2.0	-0.9	0.6	1.3	-0.1	-13.0	-9.1	-8.7	-12.1	-6.0	-11.5	2.1	-8.0	-0.2	0.7
	CV%	7.3	2.1	4.7	4.4	3.7	2.1	3.7	3.8	2.5	7.3	8.5	5.4	4.7	6.7	8.6
HQC	Bias%	-4.7	-2.8	2.3	-1.8	-4.2	-12.1	-11.2	-7.7	-12.5	-7.1	-11.4	-4.2	-10.1	-3.0	-10.5
	CV%	3.4	3.2	4.4	4.0	3.1	1.9	4.1	9.0	7.0	3.6	8.8	2.3	13.9	5.2	6.4
		TPM					TGB					PMP				
		Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
LLOQ	Bias%	-5.2	-2.0	-10.8	-10.4	-6.4	8.0	-6.5	-17.4	-6.0	-16.7	-17.7	-6.2	8.2	-13.3	-1.6
	CV%	9.5	3.4	4.9	11.3	5.0	3.5	7.2	4.6	2.0	3.2	6.0	7.5	6.4	3.8	2.2
LQC	Bias%	-2.6	-12.2	-1.1	7.2	11.2	-7.0	-7.7	0.5	1.6	5.5	-3.5	1.5	-8.9	12.8	2.6
	CV%	8.1	6.9	8.3	10.5	13.7	3.8	4.9	5.1	5.3	5.5	2.2	7.2	8.9	2.3	2.3
MQC	Bias%	-3.0	-2.6	-0.2	0.0	-2.8	-6.1	-2.3	-2.9	-12.3	-3.3	-1.7	7.5	8.7	1.6	6.2
	CV%	4.7	9.3	6.7	7.2	9.1	4.4	4.3	4.7	4.8	3.3	3.3	4.0	3.5	5.6	2.3
HQC	Bias%	-3.2	-9.6	-9.0	-2.3	-11.6	-8.0	-4.7	-7.9	-13.3	-7.6	-2.2	3.7	6.9	-1.6	2.3
	CV%	7.2	8.6	8.8	6.9	9.0	2.4	2.4	4.8	3.7	4.9	1.3	3.1	5.0	4.7	5.4
		STP														
		Day1	Day2	Day3	Day4	Day5										
LLOQ	Bias%	2.4	7.9	-7.5	-7.4	9.8										
	CV%	2.1	5.9	17.2	12.6	12.7										
LQC	Bias%	-0.3	-5.9	1.8	6.8	13.1										
	CV%	3.0	6.8	5.4	6.4	8.1										
MQC	Bias%	-4.8	-0.5	-4.0	0.6	-3.1										
	CV%	3.6	1.9	8.0	3.7	5.2										
HQC	Bias%	-3.9	-1.7	-2.3	-0.6	-4.1										
	CV%	2.7	2.6	6.9	2.6	4.2										

Table S3. Inter-day precision and accuracy of the quantification method in human plasma for the detection of the other detected ASMs. LLOQ = lower limit of quantification, LQC = lower QC, MQC = medium QC, HQC = high QC, CNB = cenobamate, PGB = pregabalin, GBP = gabapentin, LEV = levetiracetam, ETS = ethosuximide, LTG = lamotrigine, PRM = primidone, LCS = lacosamide, ZNS = zonisamide, RUF = rufinamide, MHD = 10-monohydroxy carbazepine, BRV = brivaracetam, CBZ-EP = carbamazepine-epoxide, TPM = topiramate, TGB = tiagabine, PMP = perampanel, STP = stiripentol.

		PGB	GBP	LEV	ETS	LTG	PRM	LCS	ZNS	RUF	MHD	BRV
LLOQ	Bias%	6.0	-7.5	-4.0	-5.1	1.1	-2.6	-1.0	-8.8	1.4	-4.1	0.6
	CV%	10.0	11.4	10.1	12.0	8.1	11.5	14.3	10.9	10.7	3.4	16.0
LQC	Bias%	-4.2	-0.9	3.2	4.3	3.1	3.2	2.1	1.8	10.0	0.1	-4.1
	CV%	4.8	11.0	1.7	7.3	7.0	7.0	6.4	6.7	3.3	9.9	5.9
MQC	Bias%	3.8	-3.6	-2.6	-0.4	-2.4	-6.5	-2.7	-2.7	2.5	-0.2	-9.8
	CV%	5.2	3.5	4.3	4.2	2.3	3.4	3.4	2.8	2.4	1.3	3.1
HQC	Bias%	3.0	-6.5	-4.7	-3.2	-2.5	-7.0	-3.5	-2.9	0.8	-2.3	-10.1
	CV%	3.4	3.4	5.0	2.8	1.5	2.9	1.9	1.4	6.0	2.8	2.8
		CBZ-EP	TPM	TGB	PMP	STP						
LLOQ	Bias%	-7.9	-7.0	-10.9	-6.1	1.1						
	CV%	5.0	4.0	3.6	10.8	8.1						
LQC	Bias%	-1.2	0.5	-1.4	0.9	3.1						
	CV%	8.2	9.1	5.8	8.0	7.0						
MQC	Bias%	-3.4	-1.7	-5.4	4.5	-2.4						
	CV%	6.2	1.5	4.4	4.2	2.3						
HQC	Bias%	-7.8	-7.1	-8.3	1.8	-2.5						
	CV%	4.3	4.4	3.4	3.7	1.5						

Table S4. Demographic and therapeutic features of patients receiving CNB as co-ASMs in polytherapy at Fondazione IRCCS Istituto Neurologico Carlo Besta. CNB = cenobamate, LEV = levetiracetam, LTG = lamotrigine, PRM = primidone, LCS = lacosamide, ZNS = zonisamide, BRV= brivaracetam, TPM = topiramate, PMP = perampanel, VPA = valproic acid, CBZ = carbamazepine, PB = phenobarbital, PHT = phenytoin.

Patient	Age (years)	Gender	CNB dose (mg/day)	CNB dose (mg/kg/day)	Co-therapy
Patient 1	25	M	50	0.83	VPA, CBZ
			200	3.28	VPA, CBZ
			250	4.17	VPA
			250	4.31	VPA
			250	4.24	VPA
Patient 2	56	F	200	3.64	PB, TPM
			350	6.36	TPM
			400	7.14	TPM
			400	7.14	TPM
Patient 3	20	M	25	0.43	BRV, LCS, PMP, clobazam
			250	4.31	BRV, LCS
			275	4.82	BRV, LCS
			300	5.45	BRV, LCS
Patient 4	59	F	250	3.62	PB, BRV, LCS
			300	4.35	PB, BRV, LCS
Patient 5	39	F	200	4.00	CBZ, BRV, clobazam
			200	3.85	CBZ, BRV
Patient 6	57	F	200	3.03	LEV, PGB, LCS
Patient 7	33	M	25	0.29	LTG, LCS
			150	1.76	LTG, LCS
Patient 8	37	M	50	0.71	VPA, PB, LCS
			150	2.08	VPA, PB, LCS
Patient 9	39	F	12.5	0.11	PB, LEV, PMP
			150	1.19	PB, LEV, PMP
Patient 10	27	M	100	1.56	PHT, PB, PRM
Patient 11	75	F	25	0.56	CBZ, ZNS
Patient 12	46	M	200	2.86	BRV, TPM
Patient 13	62	M	150	1.69	LEV, LCS
Patient 14	30	M	200	3.08	BRV, LTG

Table S5. MS method transitions (in bold **quantifier ion**, in italic *qualifier ion*), parameters and retention times. Compounds are listed in order of retention time apart from CNB. CNB = cenobamate, PGB = pregabalin, GBP = gabapentin, LEV = levetiracetam, ETS = ethosuximide, LTG = lamotrigine, PRM = primidone, LCS = lacosamide, ZNS = zonisamide, RUF = rufinamide, MHD = 10-monohydroxy carbazepine, BRV = brivaracetam, CBZ-EP = carbamazepine-epoxide, TPM = topiramate, TGB = tiagabine, PMP = perampanel, STP = stiripentol.

Compound	Precursor ion (m/z)	Cone (V)	Product ion (m/z)	Collision (V)	Ion mode	Retention time (min)	Internal standard
CNB	268.02	20	154.99 <i>197.99</i>	14 10	+	3.32	Lamotrigine- 13C3,d3
PGB	160.00	34	142.00 <i>83.00</i>	10 14	+	1.00	Pregabalin-d4
GBP	172.00	38	154.00 <i>137.00</i>	11 16	+	1.01	Pregabalin-d4
LEV	171.00	22	126.10 <i>154.00</i>	14 7	+	1.08	Levetiracetam-d6
ETS	139.90	45	140.00 <i>41.90</i>	5 20	-	1.45	Ethosuximide-d3
LTG	256.10	55	211.10 <i>158.90</i>	24 30	+	1.82	Lamotrigine- 13C3,d3
PRM	219.00	25	162.20 <i>90.90</i>	12 25	+	1.97	Primidone-d5
LCS	251.10	20	91.20 <i>108.00</i>	25 8	+	2.13	Lacosamide-d3
ZNS	210.90	25	118.90 <i>147.00</i>	15 10	-	2.16	Zonisamide-d4
RUF	239.00	32	126.90 <i>100.90</i>	25 8	+	2.39	Lacosamide-d3
MHD	255.10	20	237.20 <i>194.00</i>	7 20	+	2.63	10-monohydroxy carbazepine-d10
BRV	213.10	25	168.20 <i>55.00</i>	17 35	+	2.82	Levetiracetam-d6
CBZ-EP	253.00	27	210.00 <i>180.00</i>	15 15	+	3.13	Oxcarbazepine-d4
TPM	337.97	48	337.97 <i>77.85</i>	3 32	-	3.33	Topiramate-d12
TGB	376.00	45	247.00 <i>110.90</i>	45 45	+	3.56	Tiagabine-d5
PMP	350.00	72	219.10 <i>247.00</i>	36 28	+	3.98	Tiagabine-d5
STP	217.20	35	187.00 <i>145.00</i>	15 15	+	4.22	Stiripentol-d9
Pregabalin-d4	164.10	34	146.10	10	+	0.99	
Levetiracetam-d6	177.00	22	132.10	14	+	1.08	
Ethosuximide-d3	142.90	45	143.00	5	-	1.44	
Lamotrigine- 13C3,d3	262.10	55	217.10	24	+	1.82	
Primidone-d5	224.00	25	167.00	12	+	1.94	
Lacosamide-d3	254.20	20	108.00	10	+	2.10	
Zonisamide-d4	214.90	25	122.90	15	-	2.12	

10-monohydroxy carbamazepine-d10	258.10	20	240.20	7	+	2.62
Oxcarbazepine- d4	257.00	30	240.00	12	+	3.33
Topiramate-d12	350.03	54	350.03	3	-	3.29
Tiagabine-d5	382.00	45	253.00	20	+	3.56
Stiripentol-d9	226.00	35	196.00	15	+	4.21