

VOLUMETRIC ABSORPTIVE MICROSAMPLING AS AN ALTERNATIVE TOOL FOR THERAPEUTIC DRUG MONITORING OF FIRST-GENERATION ANTI- EPILEPTIC DRUGS

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ANTI-EPILEPTIC DRUGS

First generation AEDs

e.g. carbamazepine, phenobarbital, phenytoin, valproic acid, ...



- Significant interindividual variability in pharmacokinetics (ADME)
- Narrow therapeutic ranges



Optimization and individualisation of therapy = challenging

Second generation AEDs

e.g. oxcarbazepine, topiramate, ...

Third generation AEDs

e.g. lacosamide, retigabine, ...






TDM of first generation AEDs

- Excellent tool for therapy optimization and individualization
- Helpful in maximizing safety and benefits

Most often performed on venous blood samples (whole blood, plasma or serum)



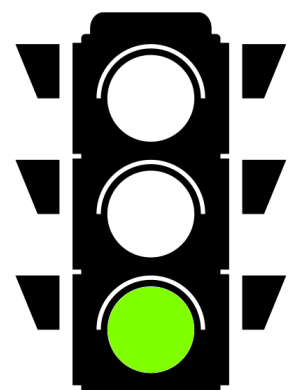
-  Invasive nature
-  Typically large amounts of blood taken
-  Need for a phlebotomist



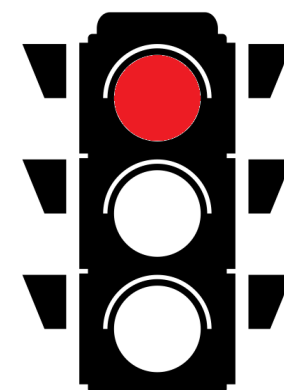
Growing interest in the use of non- and minimally invasive alternative sampling strategies

ALTERNATIVE SAMPLING STRATEGIES

One of the most commonly used = Dried blood spots



- Easy and minimally invasive (homesampling)
- Non-contagious
- Small sample volume
- Increased analyte stability
- Convenient transport and storage
- Suitable for automation



- Only small volumes available: sensitive techniques required
- Risk of contamination
- Capillary vs venous concentration
- Extensive validation required (cfr. impact of Hct, influence of spotted volume, punching site)
- Hematocrit issue



Volumetric absorptive microsampling (VAMS) devices

VAMS DEVICES

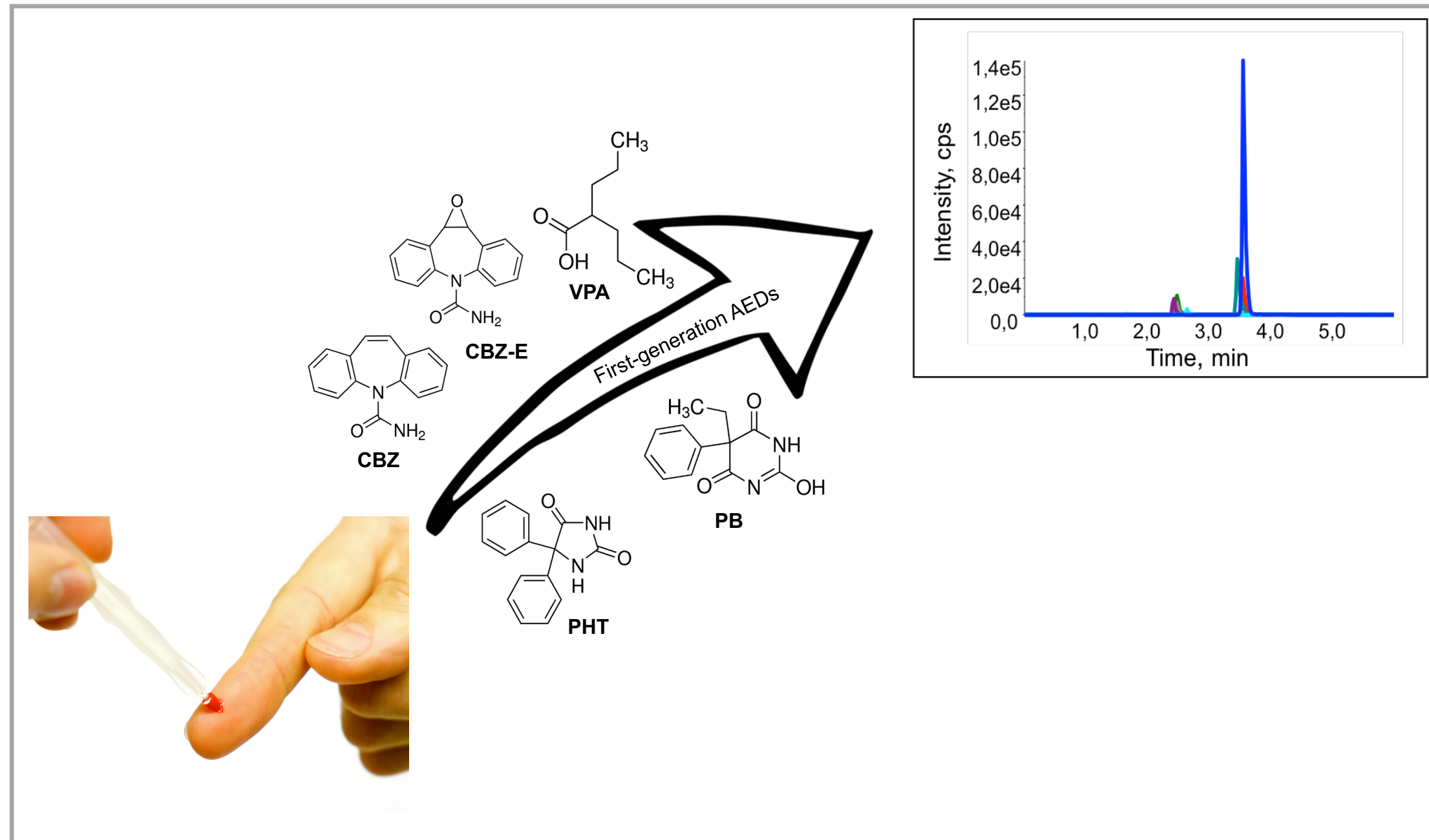
- Hydrophilic polymer tip connected to a plastic handler
- Wick up a fixed volume (approximately 10, 20 μL (or 30 μL))
- Eliminate the volumetric Hct bias associated with DBS
- Maintain the benefits associated with DBS

- Cost price
- Currently incompatible with on-line analysis systems
- Recovery may be impacted by Hct¹⁻⁴



STUDY OBJECTIVE

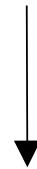
Development, validation, and application of an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC®-MS/MS) method for the determination and quantification of four AEDs and one active metabolite making use of VAMS devices.



SAMPLE PREPARATION



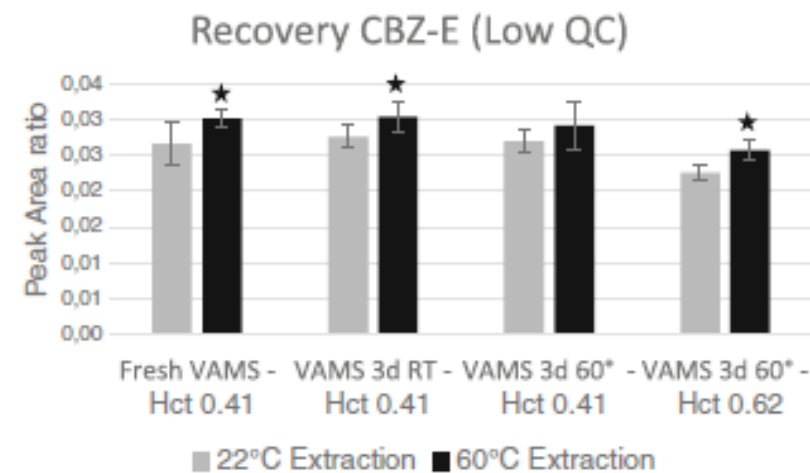
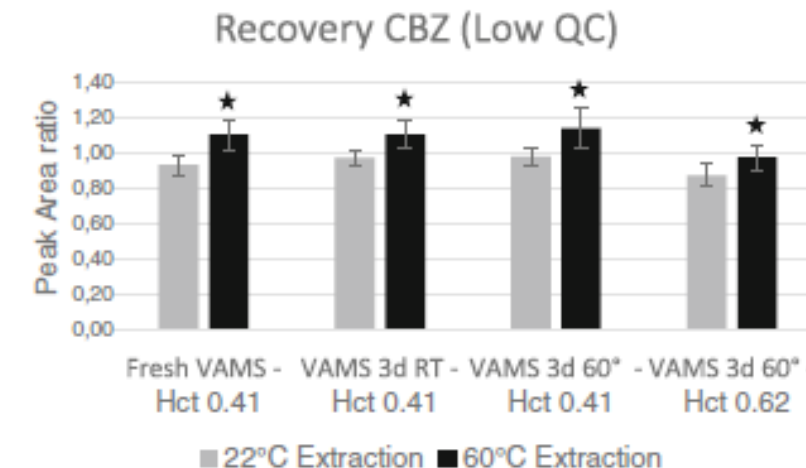
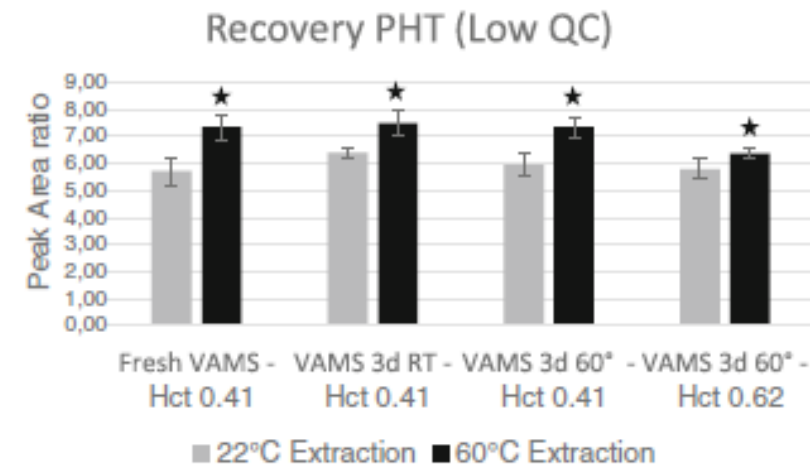
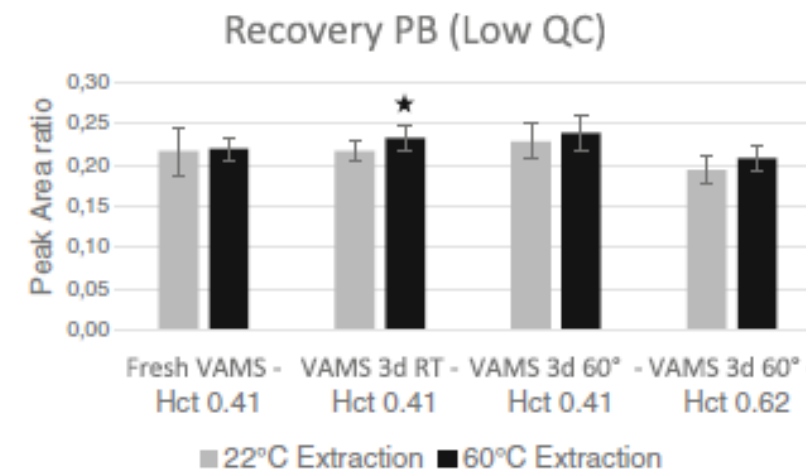
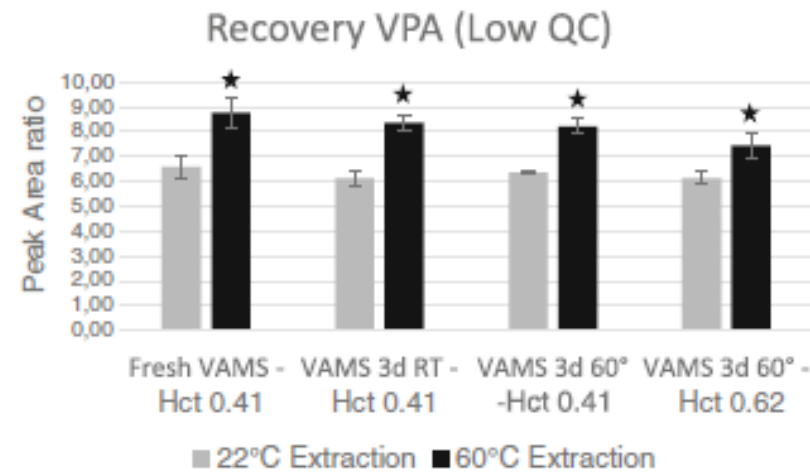
IS added to extraction solvent > no compensation for recovery issues



optimization of extraction comprehensively evaluated before validation

- Extraction at 22°C vs extraction at 60°C
- Using
 - fresh VAMS, VAMS stored 3d RT, VAMS stored 3d 60°C; QC2 - Hct 0.41
 - VAMS stored 3d 60°C; QC2 - Hct 0.62

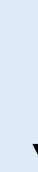
SAMPLE PREPARATION



Conclusion:

2-sample T-test (minitab®):

Overall the mean of extraction at 60°C was significantly better than the mean at 22°C (★).



Extraction of VAMS at **elevated temperature** (60°C) was chosen since it provided a significant better result in most cases.

SAMPLE PREPARATION



Preparing the VAMS



Drying for 2 hours



Removing of VAMS tips



Extraction with 100 μ L
80/20 ACN/H₂O + IS



Thermomixer:
10min; 60°C; 1000 rpm
+
Centrifugation:
10min; 10 000 g



70 μ L supernatant
+ 70 μ L H₂O



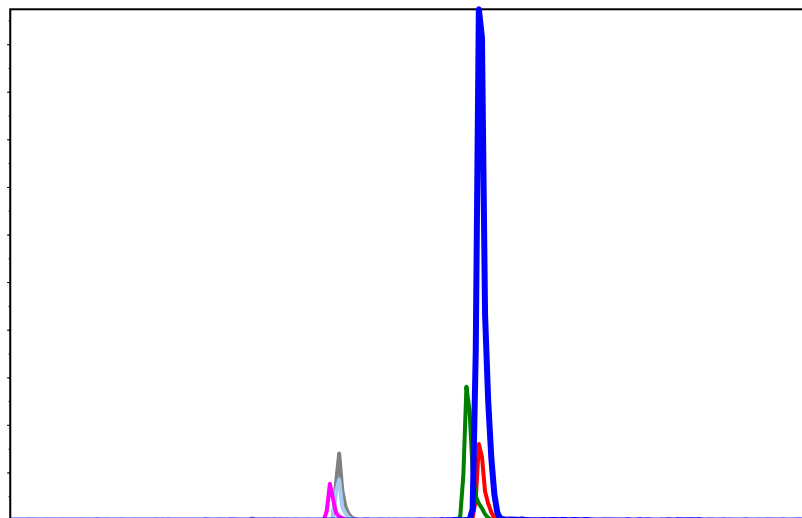
LC-MS/MS

Chromatography (Waters Acquity UPLC®)

- Column: Chromolith® reversed phase (RP)-18 endcapped (100x4.60 mm; 5 μ m)
- Mobile phase: 5mM ammonium acetate in H₂O (A) and in ACN/H₂O 95/5 (B)
- Flow: 1.4 mL/min
- Column temperature: 45°C
- Total runtime: 10 min (total run time of runs in ESI⁺ and ESI⁻ mode, washing and equilibrating)

Mass spectrometry (Sciex API 4000™)

- MRM™ mode
- Positive ionization mode (ESI⁺): CBZ, CBZ-E, OXC
- Negative ionization mode (ESI⁻): VPA, PB, PHT



METHOD VALIDATION

- Accuracy
- Precision
- Carry-over
- Selectivity
- Homoscedasticity
- Calibration model
- Stability
- Matrix effect
- Impact of Hct on Recovery



Bio-analytical specific parameters 

VAMS specific parameter

ACCURACY AND PRECISION

QC	Intra-batch precision (%RSD) (n = 4 x 2)				
	VPA	PB	PHT	CBZ	CBZ-E
LLOQ	7.47	9.76	8.60	8.76	7.67
Low	3.83	7.25	6.60	7.48	6.54
Mid	5.61	4.49	7.86	5.99	5.32
High	8.29	3.87	4.11	8.96	5.08

	Inter-batch precision (%RSD) (n = 4 x 2)				
	VPA	PB	PHT	CBZ	CBZ-E
LLOQ	7.47	9.76	8.60	8.76	7.67
Low	8.15	7.83	6.60	7.48	6.63
Mid	5.61	4.49	7.86	7.34	5.32
High	8.29	6.16	7.68	8.96	5.08

	Accuracy (%Bias) (n = 4 x 2)				
	VPA	PB	PHT	CBZ	CBZ-E
LLOQ	-15.3	-1.42	4.22	9.85	4.02
Low	18.2	-1.48	0.87	0.72	14.0
Mid	-1.14	-2.70	3.71	8.15	8.22
High	-1.32	1.51	4.84	2.01	4.97

Conclusion:

Inter- and intra-batch Precision:

The acceptance criterium was met for all compounds

Accuracy:

The acceptance criterium was met except for VPA (18.2% bias at Low QC)

Acceptance criteria:

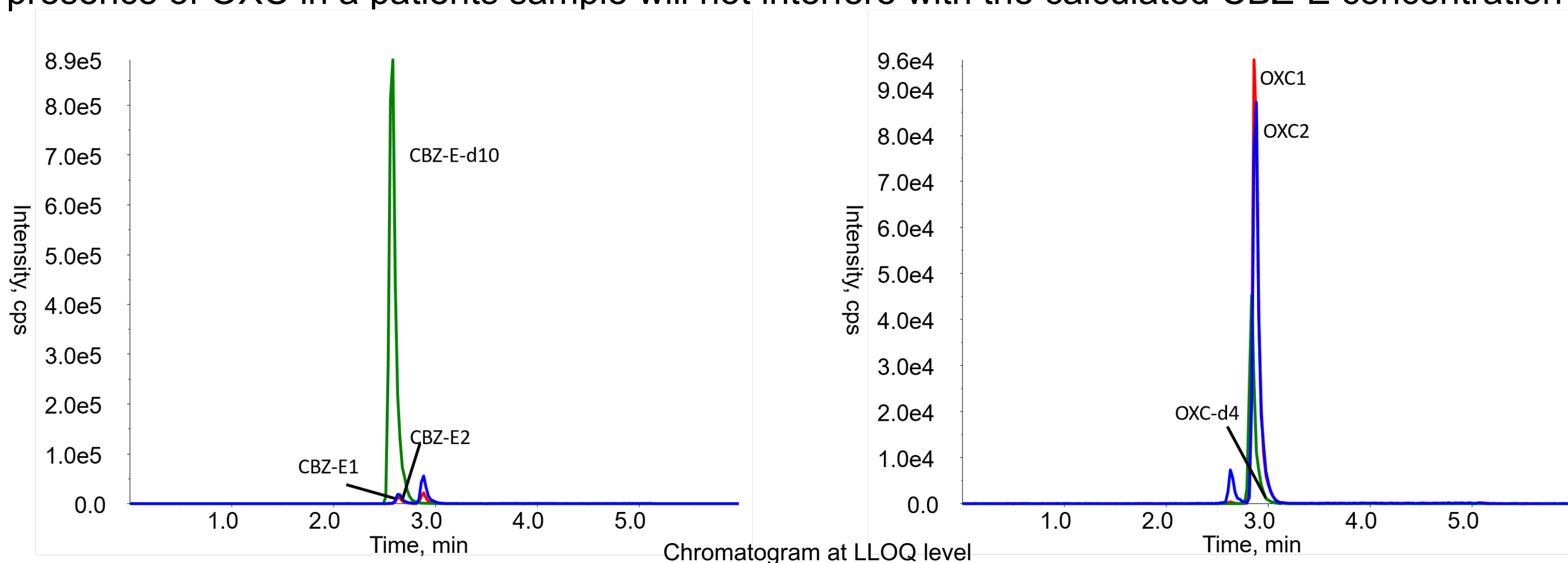
- Precision: %RSD within $\pm 15\%$ (LLOQ within $\pm 20\%$)
- Accuracy: %bias within $\pm 15\%$ (LLOQ within $\pm 20\%$)

CARRY-OVER AND SELECTIVITY

- Carry-over:
 - No carry-over detected when injecting blank samples after the highest calibrator
- Selectivity:
 - No unacceptable interferences were observed in VAMS prepared from blank blood originating from 6 different donors



Advantage: possibility to distinguish between CBZ-E and OXC (same MRM transitions): the presence of OXC in a patients sample will not interfere with the calculated CBZ-E concentration



CALIBRATION DATA

Compound	Calibration model	homoscedasticity
VPA	Quadratic regression, $1/x$	Heteroscedastic
PB	Linear regression, $1/x$	heteroscedastic
PHT	Unweighted linear regression	heteroscedastic
CBZ	Linear regression, $1/x^2$	heteroscedastic
CBZ-E	Linear regression, $1/x$	heteroscedastic

Back-calculated concentrations:

Acceptance criterium: mean back-calculated concentration within $\pm 15\%$ of the nominal value (LLOQ 20%)

Using these models > mean back-calculated concentrations did not differ more than 7% for all calibrators



STABILITY

Temp	Stability for 4 days (%difference) (n = 3)									
	VPA		PB		PHT		CBZ		CBZ-E	
	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC
RT	-6.04	-9.32	-5.34	-6.15	-8.43	4.16	-9.22	-5.51	7.45	-4.31
4°C	2.06	2.03	5.16	-0.40	-0.75	12.4	-0.31	4.69	15.9	5.29
-20°C	12.1	0.25	8.29	-6.23	1.54	9.95	0.38	-3.61	17.5	0.02
60°C	2.30	-2.33	0.03	-7.84	-4.85	3.03	-12.1	-5.12	2.87	-9.24
	Stability for 1 week (%difference) (n = 3)									
RT	11.5	0.74	-6.01	-10.5	-8.33	9.60	-13.7	-8.83	5.94	-0.10
4°C	11.8	-7.97	-7.22	-13.6	-8.37	3.75	-13.9	-13.5	6.89	-5.42
-20°C	11.8	-4.42	-3.09	-11.8	-6.12	5.20	-13.4	-14.9	4.98	-0.76
60°C	6.30	-7.97	0.56	-12.97	-10.3	2.31	-10.9	-12.3	-5.94	-15.3
	Stability for 1 month (%difference) (n = 3)									
RT	17.6	10.4	5.07	0.27	11.9	9.18	-4.58	3.15	4.01	-4.12
4°C	15.1	18.0	7.00	5.88	11.8	7.75	-3.64	-0.91	11.4	-1.66
-20°C	15.5	17.6	13.2	7.36	9.46	7.38	-1.75	1.67	5.11	-0.22
60°C	16.3	28.1	15.5	6.77	11.0	13.3	-10.8	-0.85	-23.6	-26.1

Conclusion:

All compounds were stable

💧 For at least 1 month when stored at RT, 4°C and -20°C

💧 For at least 1 week when stored at 60°C

Processed samples

(analytes+IS) were stable for at least 24h when stored in the autosampler (4°C)

MATRIX EFFECT

	Analyte matrix effect									
	VPA		PB		PHT		CBZ		CBZ-E	
	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC
Mean of 6 donors (%)	95	95	112	103	81.2	79.5	127	131	134	138
%RSD	3.64	3.36	5.11	4.82	2.29	2.12	10.69	8.08	12.37	11.61

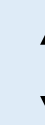
	IS-corrected matrix effect									
	VPA		PB		PHT		CBZ		CBZ-E	
	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC	Low QC	High QC
Mean of 6 donors (%)	103	101	98.3	95.7	98.6	95.6	93.1	90.4	95.4	99.0
%RSD	1.21	1.83	1.92	1.58	3.91	3.01	1.67	0.93	1.70	3.97

Conclusion:

💧 Non-IS-corrected matrix effect PHT: **ionization suppression (>15%)**

💧 Non-IS-corrected matrix effect CBZ: **ionization enhancement (>15%)**

💧 Non-IS-corrected matrix effect CBZ-E: **ionization enhancement (>15%)**

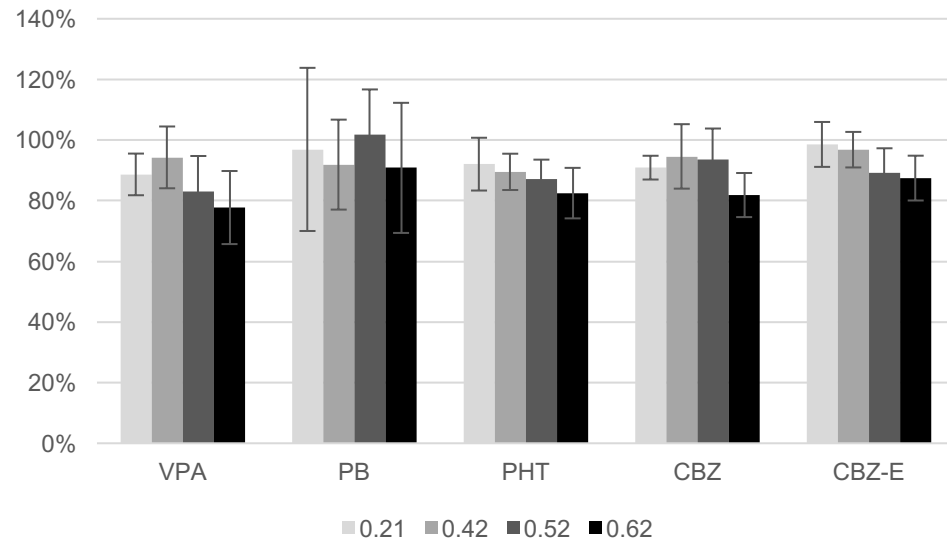


💧 IS-corrected matrix effects: all within 90-103% > IS compensates for differences in ionization

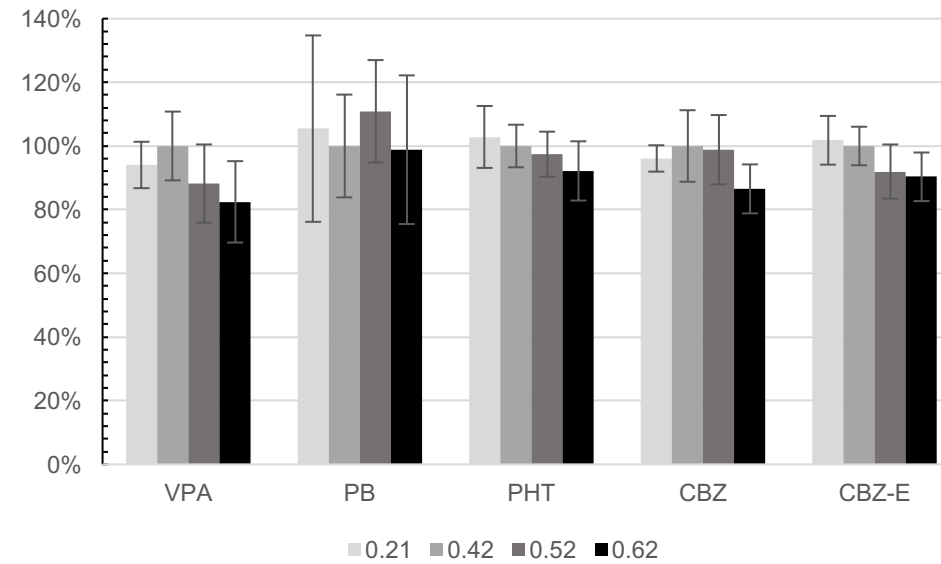
💧 %RSD of IS-corrected matrix effects <4% for all compounds: **met acceptance criterium of 15%**

IMPACT HCT ON RECOVERY (PIPETTED)

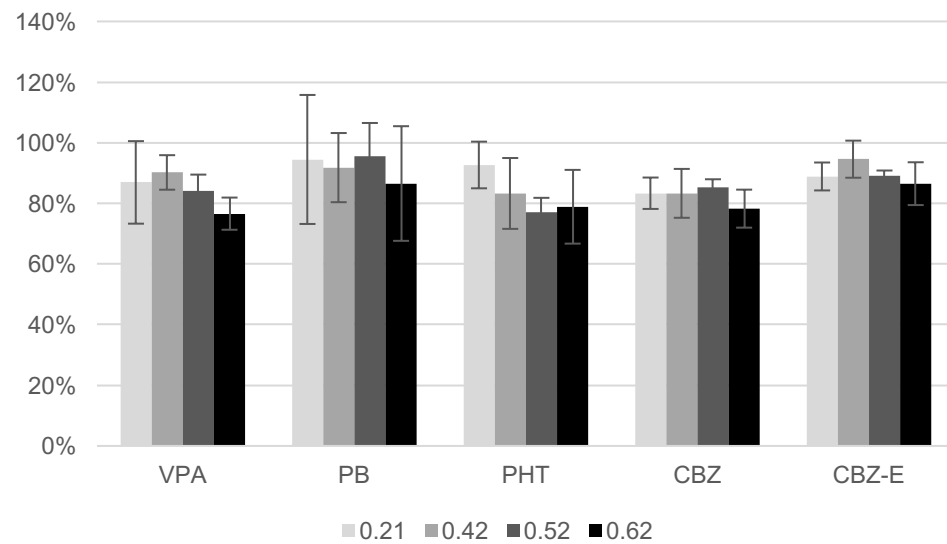
IS-compensated recovery Low QC



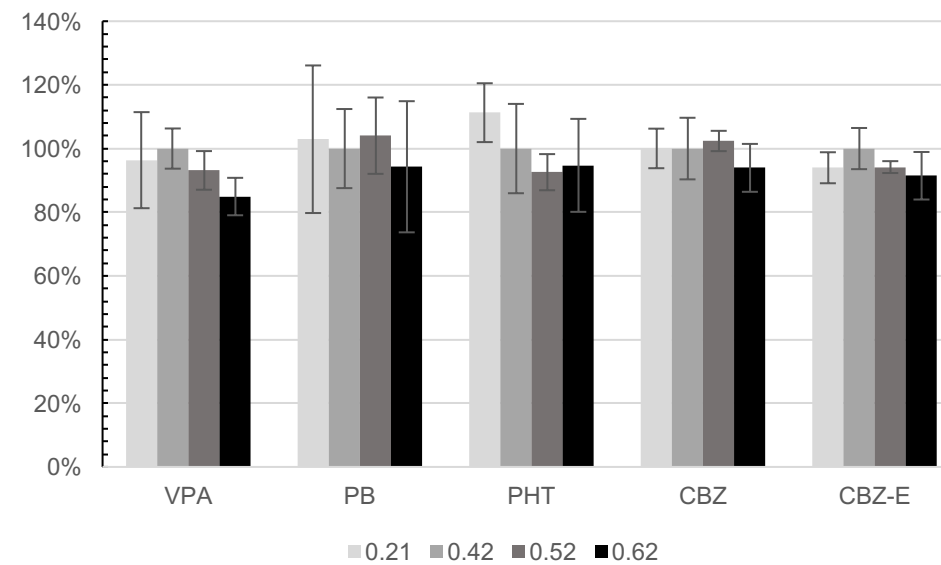
IS-compensated recovery Low QC



IS-compensated recovery High QC



IS-compensated recovery High QC



IS-compensated recovery (%)

IS-compensated recovery (%)

With the 0.42 Hct sample being normalized to 100%

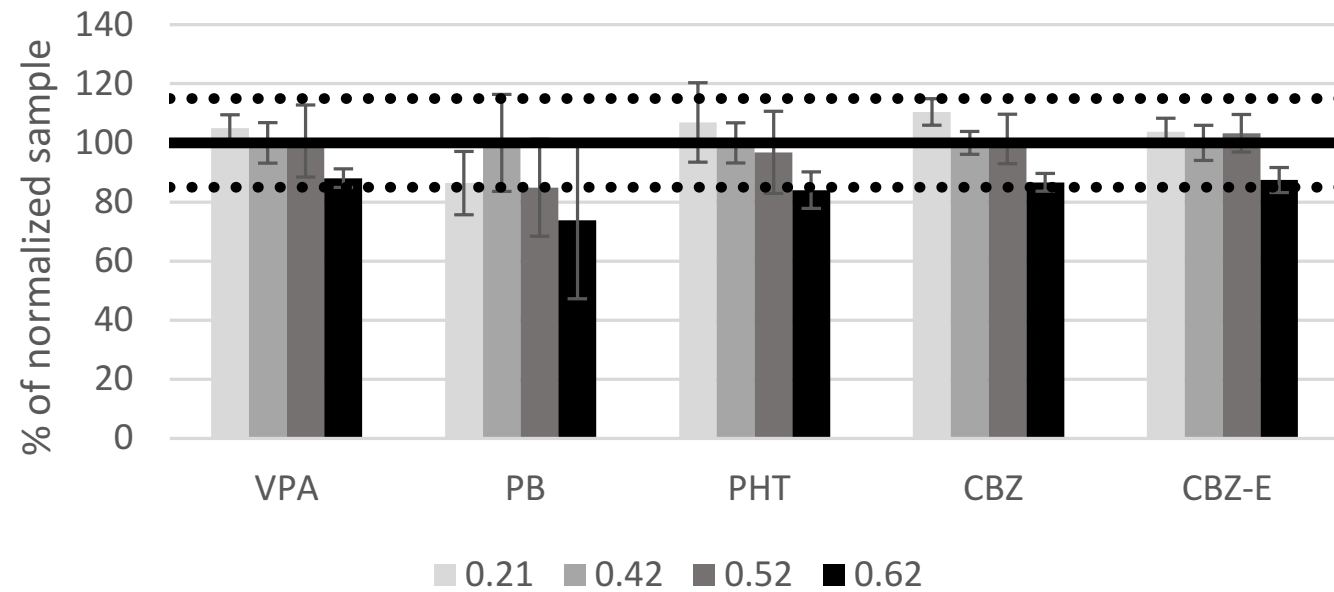
Conclusion:

- High recovery values were obtained
- When normalizing the 0.42 Hct sample to 100%, all –except for VPA– were within 15% of the 0.42 Hct sample
- VPA: the 0.62 Hct sample at low QC level differed 18% from the 0.42 Hct sample (15% at high QC level)

IMPACT HCT ON RECOVERY (WICKED)

VAMS prepared by dipping into spiked blood (QC2&4) at 4 Hct levels

Impact of Hct on recovery (dipping, Low QC)



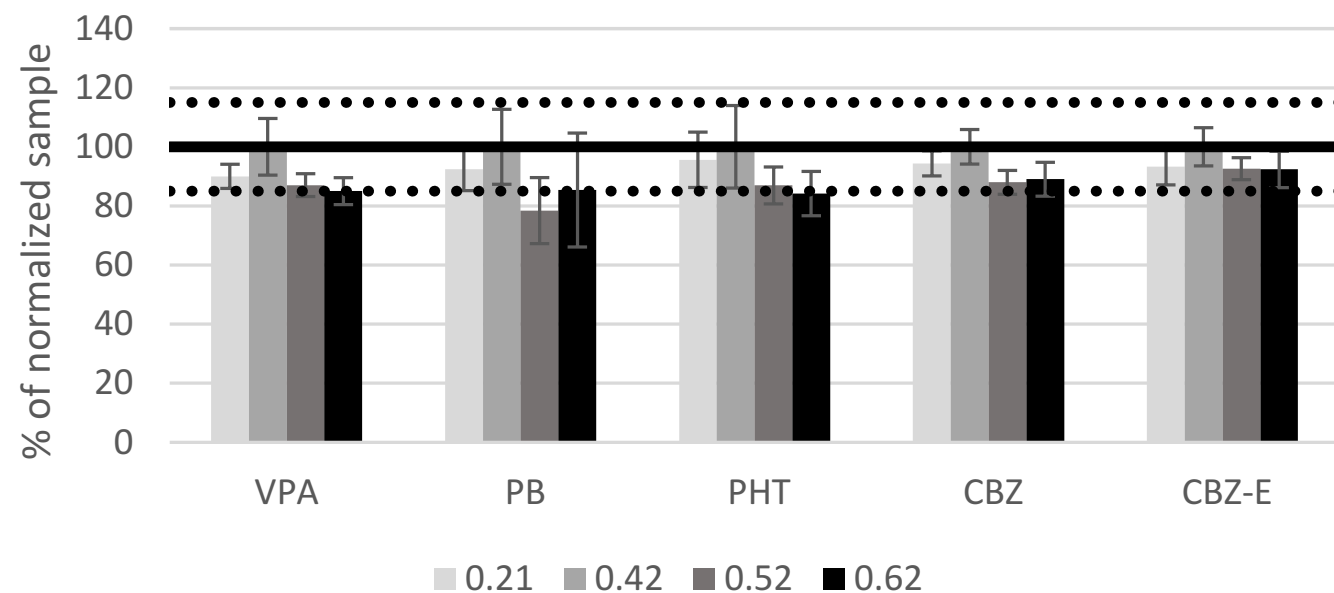
Conclusion:

💧 All were within 16% of the 0.42 sample (set to 100%), except for PB

💧 If there would be an effect, it will be limited

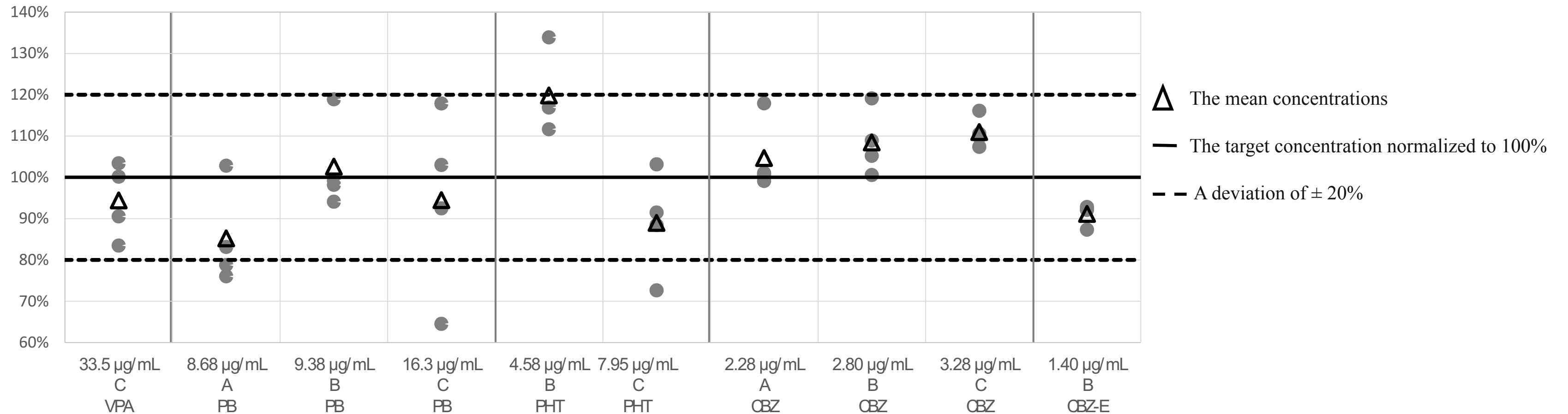
💧 However: recommendation to be cautious when analyzing samples with a Hct >0.60

Impact of Hct on recovery (dipping, High QC)



APPLICATION: EXTERNAL QUALITY CONTROL MATERIAL

- 2 sets of external quality control materials (serum)
- In order to be comparable with the calibration curve prepared in whole blood:
 - 1 on 4 dilution with whole blood: replacing 250 μL of plasma (centrifugation of 1 mL of whole blood) by 250 μL of the external quality control materials



Conclusion:

- 👉 35 out of the 40 measurements deviated less than 20% from the target value
- 👉 The mean concentrations were within $\pm 20\%$ in all cases
- 👉 The %RSD < 15% for the quadruplicates, with the exception of PB from set C (owing to one deviating value)

APPLICATION: 75 REAL-LIFE PATIENT SAMPLES

75 samples: 29 VPA, 13 PB, 13 PHT and 20 CBZ → 8 of the VPA samples & 2 of the CBZ < LLOQ → not quantified

Reference: serum concentrations (immunoassay)

	Conc VAMS (µg/mL)	Calc serum conc (µg/mL)	Serum conc (µg/mL)	% difference between calc serum conc and serum conc	VAMS conc/serum conc (%)
VPA ¹	29.3	41.9	49.5	-15.4	59.2
	56.2	80.3	79.0	1.63	71.1
	39.2	56.0	64.6	-13.3	60.7
	61.7	88.1	74.7	18.0	82.6
	30.0	42.9	50.6	-15.3	59.3
	42.7	61.0	66.8	-8.68	63.9
	44.9	64.2	61.7	3.98	72.8
	38.3	54.7	57.4	-4.78	66.7
	45.9	65.6	86.3	-24.0	53.2
	29.1	41.6	34.7	19.8	83.9
	73.5	105.0	112.3	-6.49	65.5
	42.0	60.0	63.5	-5.49	66.2
	35.1	50.1	59.7	-16.0	58.8
	28.0	39.9	30.8	29.6	90.7
	72.2	103.2	104.7	-1.46	69.0
	48.7	69.6	82.1	-15.2	59.4
	63.7	91.0	93.8	-2.99	67.9
	28.1	40.1	30.0	33.8	93.7
	51.0	72.8	100.8	-27.8	50.6
	36.5	52.2	45.1	15.7	81.0
	53.9	77.0	95.8	-19.7	56.2
			Mean±SD	Mean±%RSD	
			-2.57±17.2%	68.2±17.7%	

	Conc VAMS (µg/mL)	Calc serum conc (µg/mL)	Serum conc (µg/mL)	% difference between calc serum conc and serum conc	VAMS conc/serum conc (%)
PB ²	34.9	38.8	37.8	2.65	92.3
	7.35	8.17	8.70	-6.09	84.5
	41.6	46.2	43.6	5.96	95.4
	9.88	11.0	10.6	3.77	93.2
	8.82	9.80	8.20	19.5	107.6
	14.2	15.8	20.5	-22.9	69.3
	16.9	18.8	19.4	-3.25	87.1
	16.6	18.4	20.7	-11.1	80.1
	19.8	21.9	23.6	-7.00	83.7
	40.7	45.2	41.2	9.66	98.7
	36.9	41.0	33.6	22.1	109.9
	22.0	24.4	21.4	14.1	102.7
	27.3	30.4	27.8	9.30	98.4
			Mean±SD	Mean±%RSD	
			2.82±12.7%	92.5±12.4%	

VPA: mean of [VAMS] = 68.2±17.7% of [serum] ~ bl/pl ratio of 0.7
 PB: mean of [VAMS] = 95.5±12.4% of [serum] ~ bl/pl ratio of 0.9

1. Blood/plasma ratio 0.70 (Launiainen et al., Drug Test Anal., 2014)
 2. Blood/plasma ratio 0.90 (Morris et al., Ther Drug Monit., 1988)

APPLICATION: 75 REAL-LIFE PATIENT SAMPLES

	Conc VAMS (µg/mL)	Calc serum conc (µg/mL)	Serum conc (µg/mL)	% difference between calc serum conc and serum conc	VAMS conc/serum conc (%)
PHT ³	14.3	20.1	15.8	27.2	90.5
	5.47	7.70	5.32	44.7	102.8
	8.31	11.7	12.4	-5.65	67.0
	7.26	10.2	10.0	2.00	72.6
	7.23	10.2	9.10	11.9	79.5
	8.13	11.5	13.4	-14.5	60.7
	10.3	14.6	13.6	7.01	76.0
	4.00	5.63	6.00	-6.14	66.6
	4.34	6.11	5.20	17.4	83.4
	8.24	11.6	9.50	22.2	86.8
	6.61	9.31	7.80	19.3	84.7
	10.6	14.9	13.3	12.4	79.8
	4.23	5.96	6.70	-11.0	63.2
				Mean±SD	Mean±%RSD
				9.76±16.9%	78.0±15.4%

	Conc VAMS (µg/mL)	Calc serum conc (µg/mL)	Serum conc (µg/mL)	% difference between calc serum conc and serum conc	VAMS conc/serum conc (%)
CBZ ⁴	12.2	12.0	8.60	39.5	141.9
	8.56	8.39	6.50	29.1	131.7
	5.91	5.79	5.20	11.3	113.7
	8.75	8.58	6.40	34.1	136.7
	2.71	2.66	2.30	15.7	117.8
	11.7	11.5	13.1	-12.2	89.3
	6.51	6.38	5.40	18.1	120.6
	9.36	9.18	10.6	-13.4	88.3
	6.97	6.83	7.70	-11.3	90.5
	7.17	7.03	6.30	11.6	113.8
	6.56	6.43	6.80	-5.40	96.5
	4.76	4.67	5.00	-6.61	95.3
	7.87	7.72	5.40	42.9	146
	4.11	4.03	3.20	26.1	128.6
	10.6	10.4	8.80	18.2	120.6
	14.4	14.1	11.7	20.3	122.7
	10.3	10.1	8.00	26.1	128.6
	7.79	7.64	6.10	25.2	127.7
				Mean±SD	Mean±%RSD
				15.0±18.0	117.2±15.6

PHT: mean of [VAMS] = 78.0±15.4% of [serum] ~ bl/pl ratio of 0.71
 CBZ: mean of [VAMS] = 117.2±15.6% of [serum] ~ bl/pl ratio of 1.02

- Blood/plasma ratio 0.71 (Morris et al., Ther Drug Monit., 1988)
- Blood/plasma ratio 1.02 (Houts., Principles and practice of immunoassay, 1991)

CONCLUSION

- 💧 An **LC-MS/MS method** for the determination and quantification of 4 AEDs and 1 active metabolite, making use of VAMS, was **developed**.
- 💧 The final method was **extensively validated**, including both bioanalytical and VAMS-specific parameters.
- 💧 Overall the **pre-set acceptance criteria** were met.
- 💧 **Thorough optimization of the extraction procedure** helped enabling a Hct-independent recovery.
- 💧 Application on **external quality control materials** and on **real-life patient samples** demonstrated the **validity** and the **applicability** of the developed procedure.

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