What Happened with My Topiramate?

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Problem

Our Antiepileptics in Serum by LC-MS/MS assay measures 7 antiepileptic drugs: gabapentin (GBP), lacosamide (LCS), levetiracetam (LEVE), pregabaline (PGB), **topiramate (TOPI)**, vigabatrine (VGB), zonisamide (ZNS).

Method was validated with in-house spiked calibrators. Recently, the calibration curve stopped meeting acceptance criteria, which necessitated the purchase of a commercial calibrator set (ClinCal®-Antiepileptics 5 calibrators, Recipe).

When we changed calibrators from in-house prepared to commercial calibrators the measured concentration for TOPI was nearly twice as high as the reported mean concentrations in the ClinCheck®-Antiepileptics 2 Controls Level I and II in current use. The control values for all other analytes were within the acceptance criteria.

	Level I [mg/l]		Level II [mg/l]	
	Measured	Mean (Control Range)	Measured	Mean (Control Range)
GBP	3,02	2,90 (2,03 – 3,77)	16,2	17,9 (13,5 – 22,4) ✓
LCS	4,10	3,91 (2,74 – 5,09)	11,1	11,2 (8,37 – 14,0) ✓
LEVE	9,97	9,54 (6,67 – 12,4)	23,0	23,5 (17,6 – 29,4) ✓
PGB	1,66	1,83 (1,28 – 2,38)	5,31	6,16 (4,62 – 7,71) ✓
TOPI	**4,07**	**2,79 (1,96 – 3,63)**	**13,4**	**8,49 (6,37 – 10,6)** ✗
VGB	4,81	4,40 (3,08 – 5,72)	18,0	17,5 (13,2 – 21,9) ✓
ZNS	10,2	9,49 (6,64 – 12,3)	24,9	21,7 (16,3 – 27,1) ✓

Method information

There are two methods for antiepileptics in current use in our laboratory:

Antiepileptics in Serum assay (AED assay), using a 7-min LC gradient and measuring all analytes in positive ESI mode, and an abbreviated antiepileptics method for LEVE and TOPI only (LEVE TOPI assay), using a 4-min LC gradient, with TOPI being measured in negative ESI mode.

All other parameters (below) are common to both methods.

Sample preparation:

- 25µl of sample (calibrator, control, serum) was precipitated with 125µl protein precipitation solution (containing internal standards in acetonitrile, c=1mg/l)

Instrumentation and chromatographic conditions

- Agilent 1290 Infinity LC System
- Agilent 6460 Triple Quadrupole LC/MS System
- MP-A: 0,1% Formic acid in water
- MPB: acetonitrile
- Flow rate: 0.35 ml/min
- Column: Kinetex®-2.6µm PFP 100Å LC Column 50x3 mm
- Column oven temperature: 35 °C
- Injection volume: 1.5 µl

MRM transitions for TOPI and TOPI-d12

ESI+	Precursor	Quant	Qual
TOPI	340	264	184
TOPI-d12	352	270	288
ESI-	**Precursor**	**Quant**	**Qual**
TOPI	338	78	96
TOPI-d12	350	78	96

Troubleshooting steps taken

- **We investigated whether there were any accidental changes or errors made in either the acquisition or the quantitation method.**
  - We did not find any.
- **We considered that the commercial calibrator concentrations may not have been assigned correctly.**
  - When inquiring with a Recipe representative in the Czech Republic, we were told that no other laboratory using ClinCal®-Antiepileptics 5 calibrators reported any issue.
- **We tested the ClinCal®-Antiepileptics 5 calibrators with our abbreviated antiepileptics LEVE TOPI assay (negative ESI mode for TOPI)**
  - We found that TOPI concentrations in ClinCheck®-Antiepileptics 2 controls were in range.
  - Therefore, we considered that the problem could be matrix related.
- **We ran a comparison of a series of samples (10 patient, 2 QC, 2 EQAS samples) under different conditions (5 assays which differed in gradient and ESI mode, see below)**

I	LEVE TOPI assay (in current use)	ESI- for TOPI
II	AED assay 0.3 ml/min	ESI+
III	AED assay 0.35 ml/min (in current use)	ESI+
IV	AED assay pos neg 0.3 ml/min	ESI-
V	AED assay pos neg 0.35 ml/min	ESI-

- **We evaluated TOPI results using ClinCal®-Antiepileptics 5 calibrators as well as an older lot of in-house calibrators prepared before the calibration curve stopped meeting acceptance criteria.**
  - TOPI results in the table below are shown in mg/l.

Samples	Calibration used for evaluation	Assay I	Assay II	Assay III	Assay IV	Assay V
LEVEL I	in-house	2.36	2.50	2.68	2.33	2.40
	commercial	2.78	3.15	4.07	2.96	3.11
LEVEL II	in-house	8.14	7.66	8.89	7.52	8.25
	commercial	9.53	9.82	13.38	9.63	10.48
Patient 1	in-house	2.41	2.40	2.55	2.49	2.48
	commercial	2.83	3.02	3.72	3.16	3.15
Patient 2	in-house	4.38	4.61	4.87	5.05	4.14
	commercial	5.14	5.88	7.19	6.42	5.27
Patient 3	in-house	4.41	4.34	5.03	4.44	4.49
	commercial	5.17	5.53	7.45	5.67	5.71
Patient 4	in-house	7.07	7.07	7.79	7.17	6.79
	commercial	8.27	9.05	11.52	9.18	8.63
Patient 5	in-house	2.13	2.27	2.44	1.93	2.07
	commercial	2.51	2.85	3.57	2.45	2.63
EQAS 1	in-house	2.13	2.27	2.44	1.93	2.07
	commercial	2.51	2.85	3.57	2.45	2.63
EQAS 2	in-house	10.54	10.72	11.39	10.70	11.84
	commercial	12.32	12.78	16.95	12.73	12.05

Outcome

- Our troubleshooting revealed that TOPI signal in our 7-analyte Antiepileptics in Serum assay was affected by the ClinCal®-Antiepileptics 5 calibrators matrix.
- When in-house calibrators were used, results for TOPI in ClinCheck®-Antiepileptics 2 controls were in range and consistent for all five assay variations.
- With ClinCal®-Antiepileptics 5 calibrators, we observed significantly higher TOPI concentrations only when using Assay III, the current 7-analyte Antiepileptics in Serum assay measuring TOPI in ESI+ mode with a flowrate of 0.35 ml/min.
- For patient and EQAS samples the same trend was observed.

- To solve the problem with TOPI quantification, we changed the MRM transitions for TOPI in the Antiepileptics in Serum assay.
- TOPI is now measured in negative ESI mode and there is no difference observed in results when evaluated with spiked or commercial calibrators.