ARK™ LACOSAMIDE ASSAY ON BECKMAN AU480® CHEMISTRY ANALYZER

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Introduction

ABSTRACT

Background

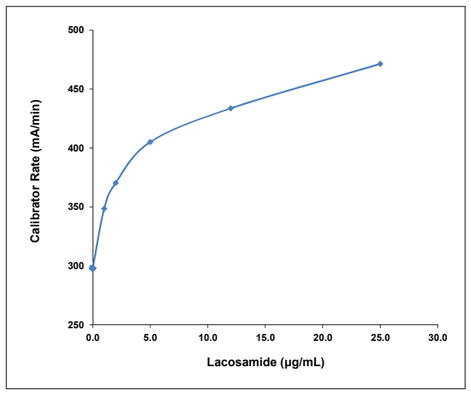
Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Lacosamide (LCM) is a third generation antiepileptic drug (AED). Typical serum levels are 7.9 ± 4.9 µg/mL (31.4 ± 19.5 µmol/L). Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of lacosamide is described.

Methods

The ARK™ Lacosamide Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.0, 1.0, 2.0, 5.0, 10.0 and 25.0 µg/mL) and 3 controls (1.5, 7.0 and 15.0 µg/mL). The performance of the ARK assay was evaluated on the Beckman AU480 Automated Clinical Chemistry Analyzer. Precision, limit of quantitation, recovery, specificity and method comparison were studied.

Calibration Curve

The assay system uses 6 calibrators which contain lacosamide at concentrations of 0.0, 1.0, 2.0, 5.0, 10.0 and 25.0 µg/mL.



Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A3. Tri-level controls containing lacosamide were assayed in quadruplicate twice a day for 5 days. Mean determinations of lacosamide, standard deviation (SD) for within-run, between-day, and total coefficients of variation (% CVs) were calculated.

			Within Run		Between Day		Total	
Sample	N	Mean (µg/mL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
QC Low (1.5 µg/mL)	40	1.54	0.083	5.4	0.047	3.1	0.118	7.7
QC Mid (7.0 µg/mL)	40	7.15	0.302	4.2	0.136	1.9	0.315	4.4
QC High (15.0 µg/mL)	40	15.26	0.896	5.7	0.263	1.7	1.170	7.7

Lower Limit of Quantitation

Limit of quantitation was evaluated according to CLSI/NCCLS EP17-A2. Pooled human serum was supplemented with known amounts of lacosamide and assayed 40 times. The LLOQ of the ARK Lacosamide Assay is defined as the lowest concentration for which acceptable inter-assay precision (≤20% CV) and recovery (±15%) is observed. The criteria of LLOQ were met at 0.6 µg/mL; the precision was 11.3% CV and the recovery was 86.5%.

Conc. Tested (µg/mL)	Mean (µg/mL)	RMS SD	CV (%)	Recovery (%)	N
0.50	0.42	0.05	11.1	83.2	40
0.60	0.52	0.06	11.3	86.5	40
0.70	0.67	0.07	11.2	95.5	40

Analytical Recovery

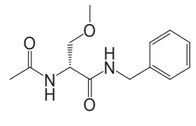
Accuracy (analytical recovery) was performed by adding certified lacosamide (Cerilliant) into human serum negative for lacosamide. Test sample concentrations were 0.70, 1.75, 3.00, 6.00, 8.00, 12.00, and 20.00 µg/mL. Two analytical runs of three replicates of each sample were assayed. The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated. The recovery of lacosamide ranged from 87.9% to 106.7%.

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
0.70	0.62	0.05	8.2	87.9
1.75	1.74	0.15	8.5	99.1
3.00	3.15	0.18	5.8	105.0
6.00	6.32	0.23	3.7	105.3
8.00	8.03	0.61	7.6	100.4
12.00	12.37	1.05	8.5	103.1
20.00	21.35	1.58	7.4	106.7

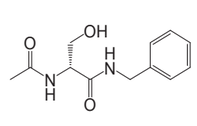
Crossreactivity

The major metabolic pathway of lacosamide is an enzymatic dealkylation of lacosamide yielding
O-desmethyl lacosamide. The metabolite has no known pharmacological activity. The lacosamide metabolite, O-desmethyl lacosamide was tested in the immunoassay for potential cross reactivity. It is known that O-desmethyl-lacosamide, is approximately 10% of that of lacosamide.

A high concentration of the metabolite O-desmethyl lacosamide was spiked into normal human serum (5.0, 10.0, and 20.0 µg/mL) in the presence of lacosamide (5.0 or 10.0 µg/mL) and assayed along with a serum control of lacosamide. The mean of 6 replicates was used to calculate the percentage crossreactivity.



Lacosamide



O-desmethyl Lacosamide

Lacosamide (µg/mL)	O-desmethyl Lacosamide (µg/mL)	Percent Crossreactivity (%)
5.0	5.0	-0.6
5.0	10.0	1.3
10.0	10.0	7.4
10.0	20.0	2.7

Proficiency Samples

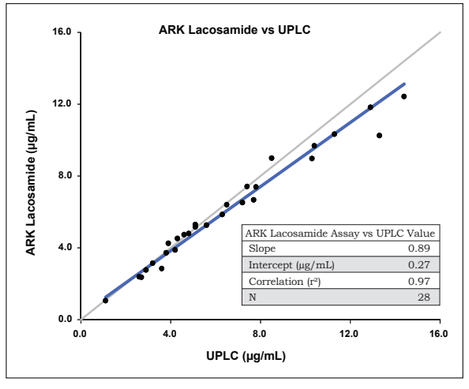
Eleven (11) proficiency samples (AE2) from LGC Standards (LGC Standards Proficiency Testing, 1 Chamberhall Business Park, Chamberhall Green, Bury, BL9 0AP, UK) were tested using ARK Lacosamide Assay. All ARK values fell in the Consensus Expected Range of proficiency samples.

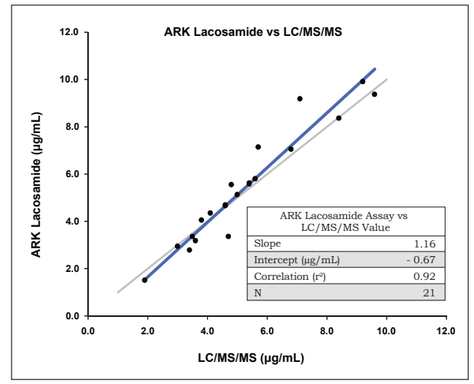
Sample ID	Assigned Value (µg/mL)	Consensus Expected Range (µg/mL))	ARK Value (µg/mL)	% Assigned Value
R 103	17.55	14.96-20.14	15.55	88.6
R 104	12.20	10.44-13.96	12.18	99.8
R 105	4.90	4.02-5.78	4.55	92.9
R 106	7.08	5.97-8.19	6.64	93.8
R 107	16.40	14.0-18.80	15.10	92.1
R 108	8.72	7.40-10.04	8.30	95.2
R 109	21.50	18.19-24.81	20.67	96.1
R 111	10.51	8.98-12.04	10.70	101.8
R 136	23.12	19.52-26.72	22.46	97.1
R 137	9.94	8.48-11.40	10.23	102.9
R 138*	26.60	22.05-31.15	26.56	99.8

*Sample was diluted by factor of 4 and tested.

Method Comparison

Forty-nine (49) leftover de-identified human serum specimens from patients treated with lacosamide were obtained from TEMA RICERCA Srl (Matteo Conti and Edit Pierini, Central Laboratory Policlinico S.Orsola-Malpighi, Bologna, Italy). These specimens were analyzed in singlicate by the ARK Lacosamide Assay. Twenty-eight specimens (28) were compared to Ultra Performance Liquid Chromatography and twenty-one (21) specimens were compared to LC-MS/MS.





Conclusions

An assay was developed that measures lacosamide in human serum with excellent precision at low concentrations. ARK Lacosamide reagents, calibrators and controls are in liquid form ready-to-use. Ability to measure trough levels of lacosamide accurately and with fast turn-around time will enable clinically useful, routine monitoring of lacosamide.

PROPOSED INTENDED USE

The ARK Lacosamide Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of lacosamide in human serum on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of lacosamide to help ensure appropriate therapy.

REGULATORY STATUS

The performance characteristics of the ARK Lacosamide Assay have not been established. The assay has not been cleared by the U.S. FDA for in vitro diagnostic use.