Comparison of the ARK Lacosamide Assay with an LC-MS/MS assay for the quantification of LACOSAMIDE concentrations in human serum/plasma samples

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INTRODUCTION

Lacosamide is a third generation antiepileptic drug, available in Italy since 2008. Lacosamide is a functionalized amino acid compound specifically synthesized as an antiepileptic drug to use as add-on therapy for partial-onset seizures from the age of 16 with antinociceptive and neuroprotective activities.

Monitoring of antiepileptic drug concentrations in plasma may provide important information to customize the therapy for each patient. Our lab is performing therapeutic drug monitoring of anti-epileptics drugs from at least 30 years.

This study was designed to evaluate the performances of a new immunoassay provided by TEMA RICERCA and manufactured by ARK Diagnostica, Inc. (USA) for lacosamide quantification in serum/plasma using a validated high performance liquid chromatography-mass spectrometry (LC-MS/MS) method as reference method.

MATERIALS AND METHODS

Samples collected from patients (n=72): blood samples were collected in tubes containing separating gel and clotting agent and centrifuged (3500 rpm for 15 minutes at RT).

Instruments: LC-MS/MS apparatus is composed by Trascend TLX-1 HPLC system and TSQ Quantum Access Mass (Thermo Scientific®).

ARK Immunoassay was performed on Abbot Architect System and ARK Lacosamide Assay Kit was adapted according to manufacturer instruction.

Antiepileptic drugs-deuteurated Internal Standards Kit for LC-MS/MS is provided by Alifax-Eureka.

RESULTS

Precision profile is described in table 1. and was determined according to SIBIOC guidelines concerning method comparison. We performed QC and precision run (3 samples per 3 replicates each run) twice a day during 5 days (figure 1).

Within-run precision was determined with 20 replicates of each control level and results are shown in table 2.

Once established the performance assay real sample were compared: regression analysis and Bland Altman plot are shown in figures 2 – 3.

	QC 1 1,65 mg/L	QC 2 7,54 mg/L	QC 3 16,23 mg/L
CV% Intra run	3,66	3,02	3,98
CV% Intra day	3,03	2,94	3,74
CV% Between day	3,86	1,41	1,29
CV% Total	7,88	8,24	6,18

Tab. 1: precision profile

PRECISION PROFILE

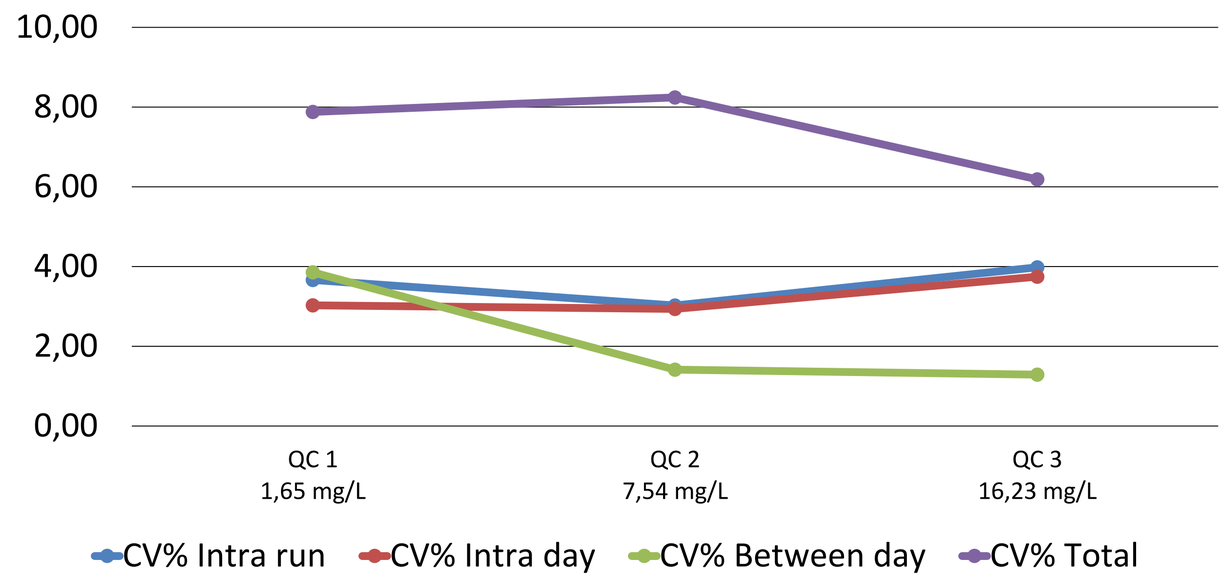

Fig. 1

	QC Low 1,5 mg/L	QC Mid 7,0 mg/L	QC High 15,0 mg/L
Mean (n=20)	1,635	7,310	16,190
SD	0,051	0,278	0,606
%CV	3,128	3,805	3,745

Tab. 2: Within-run precision

ARK IMMUNOASSAY VS LC-MS/MS METHOD

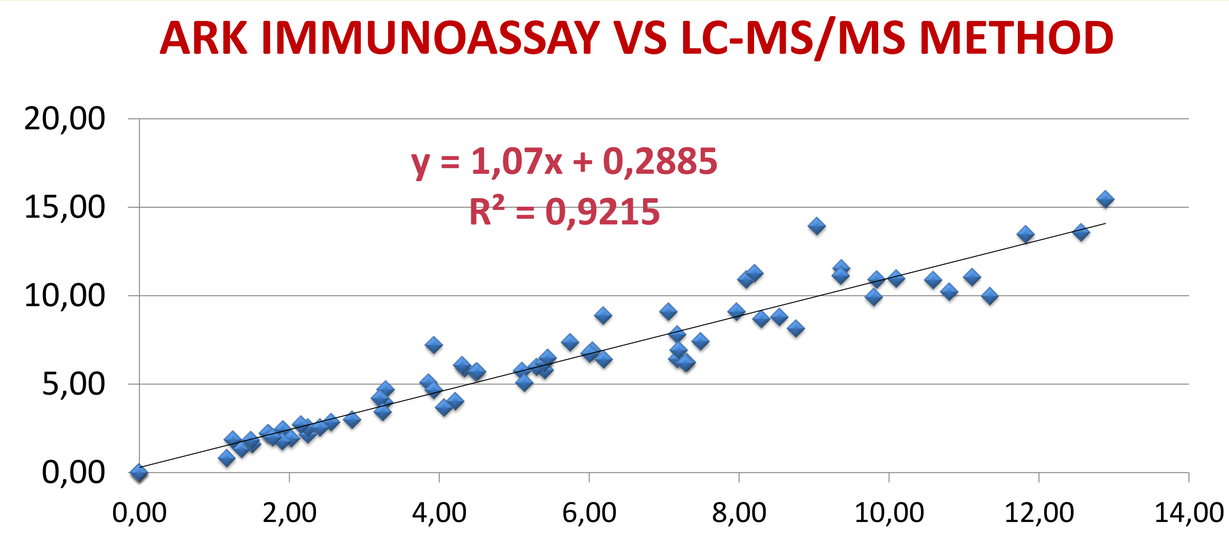

Fig. 2

BLAND ALTMAN

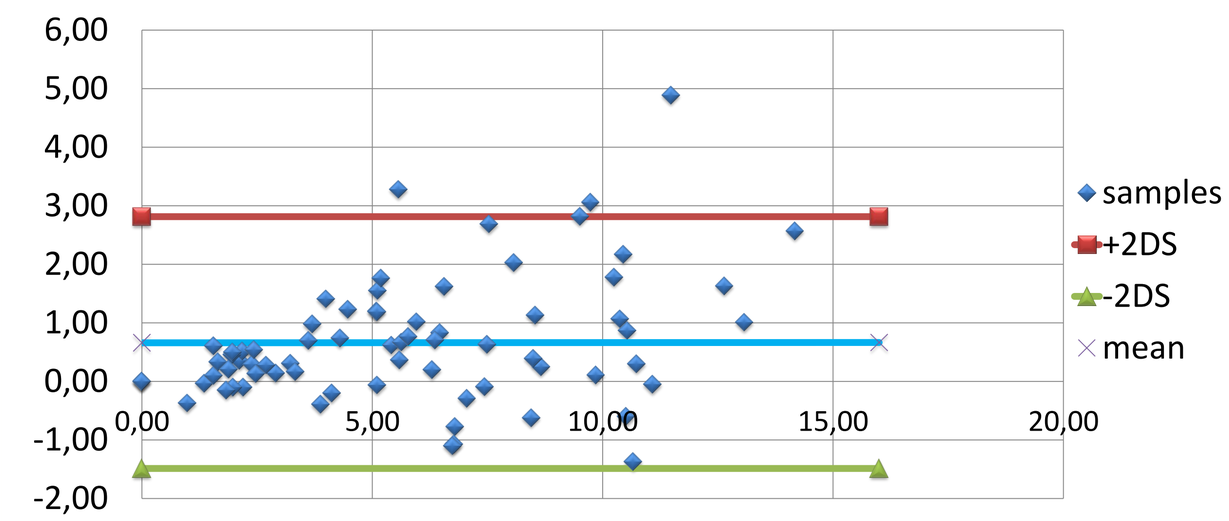

Fig. 3

CONCLUSIONS

In our hands ARK assay revealed a very good precision, repeatability and robustness. Calibration curve are very stable, for at least 2 months. It is very fast compared to other similar test with a run time of about 6 minutes.

Overall, the results show that despite a slight overestimation of the actual value of drug concentration in real samples in the higher concentration levels, this assay can be used and is reliable in monitoring lacosamide concentration in the clinical practices for the majority of patients.

It could be, therefore, considered as a viable alternative to HPLC or LC-MS/MS methods for routine lacosamide monitoring in the clinical practice.

Krasowski MD, McMillin GA. "Advances in anti-epileptic drug testing." *Clin Chim Acta.* 2014 Sep 25;436:224-236.

Patsalos PN, Berry DJ. Pharmacotherapy of the third-generation AEDs: lacosamide, retigabine and eslicarbazepine acetate. *Expert Opin Pharmacother* 2012;13:699–715.

Vidali M, et al. «Protocollo per la comparazione di due metodi analitici di laboratorio», *Biochimica clinica*, 2016, vol. 40, n. 2