Analytical Validation of an Automated Dried Blood Spot Desorption LC-MS/MS Method for Levetiracetam and Lamotrigine

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ABSTRACT

BACKGROUND

Levetiracetam and lamotrigine are antiepileptic drugs used in the treatment of tonic-clonic seizures but require monitoring due to their narrow therapeutic ranges. Traditional monitoring methods utilize serum samples, requiring frequent venipunctures for patients. Dried blood spots (DBS) are an alternative specimen type which can easily be collected at home. However, DBS require laborious sample preparation before measurements using LC-MS/MS, limiting their use in high test volume laboratories. Herein we report a novel LC-MS/MS method that allows for the coincidental quantification of levetiracetam and lamotrigine from DBS utilizing direct inline flow through desorption technology which requires no sample preparation.

OBJECTIVE

The goal of this study was to validate an LC-MS/MS method utilizing inline flow-through desorption technology to quantify levetiracetam and lamotrigine from DBS samples .

METHODS

DBS were prepared by spiking bovine whole blood with levetiracetam and lamotrigine and depositing these mixtures onto dried matrix cards. Direct inline matrix desorption and chromatographic separation were achieved using a Spark Holland DBSA module with CTC autosampler coupled to a Thermo Scientific TLX-1 (DBSA-TLX-1). Samples were purified online using a Cyclone-P (1 x 50 mm) TurboFlow column and then eluted onto an Accucore™ aQ (3 x 150 mm) analytical column for further chemical separation. The HPLC was coupled to a Thermo Scientific TSQ Altis Plus triple quadrupole mass spectrometer for ion selection and detection. Sample quantification was performed using isotopically labeled internal standards and by comparison to an external calibration curve. Twenty runs over the course of ten days were used to determine accuracy and precision.

RESULTS

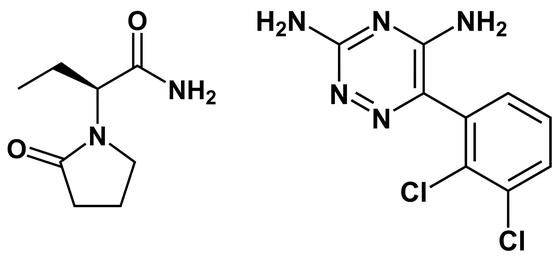
Calibration curves for both anticonvulsants maintained a linear response (R^2>0.99) which encompassed their respective therapeutic windows. Within-run precision varied with %CVs of 3.6-7.8% and between-run precision varied with %CVs of 4.5-9.7% for both drugs. Passing-Bablok regression analysis of accuracy studies revealed excellent correlations (R^2=0.99) for both drugs. Our method yielded a 14-day mean difference in concentration <±20% and <±10% compared to their originally measured concentration for levetiracetam and lamotrigine, respectively, indicating that the DBS are stable for at least 14 days .

CONCLUSIONS

Automatic flow through desorption technology in tandem with LC-MS/MS can accurately and precisely quantify levetiracetam and lamotrigine from DBS samples.

DRUG MONITORING

CLINICAL USE OF ANTICONVULSANTS



Levetiracetam C_o=12-46 μg/mL

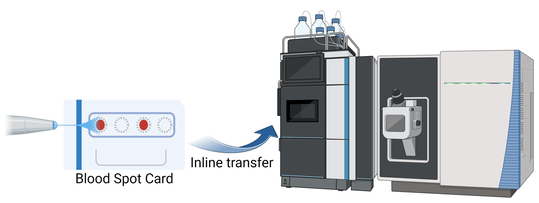
Lamotrigine C_o=2.5-15 μg/mL

- **Levetiracetam** is used for the treatment of tonic-clonic seizures, bipolar disorder, and for migraine headaches.
- **Lamotrigine** is used also used for the treatment of epilepsy and as mood stabilizer for bipolar disorder and depression.

NEGATIVE SIDE EFFECTS

- Both drugs have a narrow therapeutic window:
  - Underdosing results in suboptimal therapeutic effect.
  - Overdosing can cause toxicity.
    - Excess levetiracetam can engender anemia, neutropenia, and somnolence.
    - Excess lamotrigine can lead to vision and gastrointestinal complications.

AUTOMATIC DBS PROCESSING

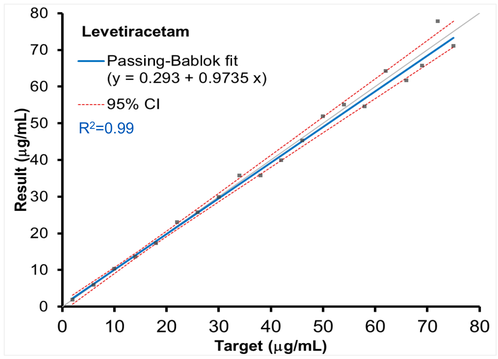


Flow Through Desorption Sample Extraction

LC-MS/MS Analysis

- Traditionally, levetiracetam and lamotrigine levels are monitored using serum samples.
- Newer methods have used dried blood spot (DBS) specimens, but sample preparation is laborious.
- The new Spark Holland DBSA module with CTC autosampler coupled to a Thermo Scientific TLX-1 (DBSA-TLX-1) allows for direct, automatic sample incorporation to LC-MS/MS without any sample preparation.

LINEARITY AND ACCURACY



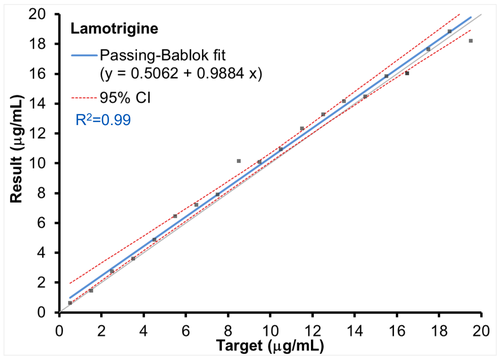


FIGURE 1: Passing-Bablok regression analysis of accuracy studies across the analytical measuring range for levetiracetam (left) and lamotrigine (right).

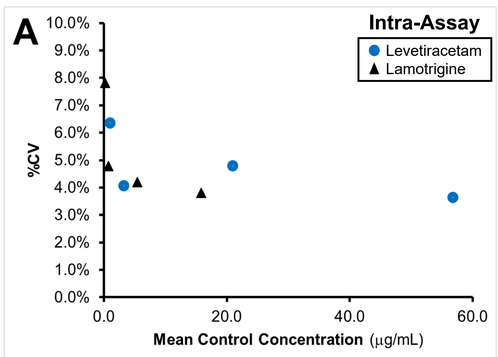
TABLE 1: DBS LC-MS/MS Detection and Quantification Limits

Analyte	Therapeutic Interval (μg/mL)	LOD (μg/mL)	% of LOQ	LOQ (μg/mL)
Levetiracetam	12-46	0.43	43%	1.01 (5.7%)
Lamotrigine	2.5-15	0.09	45%	0.20 (7.2%)

Abbreviations: DBS, dried blood spot; LOD, limit of detection; LOQ, limit of quantification; % coefficient of variation (%CV) included in parentheses.

- Linearity is maintained across the analytical measuring range and Passing-Bablok regression analysis demonstrates R^2>0.99.
- Analytical measuring range for both drugs extends well below and above the therapeutic reference interval.

PRECISION



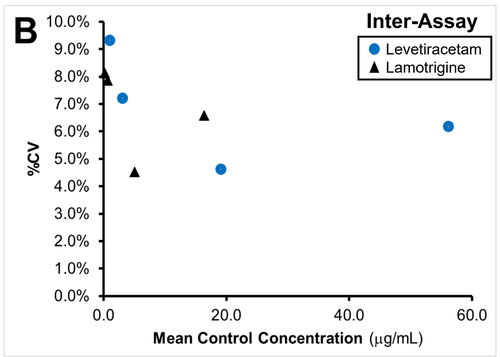


FIGURE 2: Assessment of DBS LC-MC/MS assay imprecision for **A)** Within run assay imprecision and **B)** Between run assay imprecision of levetiracetam (blue dot) and lamotrigine (black triangle). Both intra- and inter-assay imprecision were determined via 20 replicate measurements over 10 days.

- The automatic DBS flow through desorption LC-MS/MS method exhibits excellent within run and between run precision.
- %CVs for each drug at various concentrations within and between runs over 10 days are all <10%.

STABILITY

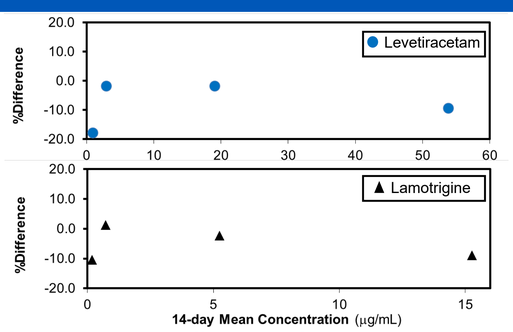


FIGURE 3: Mean difference of levetiracetam (top) and lamotrigine (bottom) concentrations in DBS over 14 days.

CONCLUSIONS

- The DBSA-TLX-1 combined with tandem MS allows for the automatic and simultaneous measurement of levetiracetam and lamotrigine from DBS without any sample preparation or pre-column extraction.
- DBS specimens have the potential to benefit patients undergoing long-term therapeutic drug monitoring.
- Additional verification of the DBSA-TLX-1 using authentic patient samples is required before this technology can be used clinically.