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# Review on bioanalytical and analytical method development of Antiepileptic racetams

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***Abstract***

Racetams are a class of medications defined by the presence of 2-pyrrolidone nucleus and are derivatives of gamma-aminobutyric acid (GABA), which acts as a major inhibitory neurotransmitter in the brain. Levetiracetam (second generation anti epileptic drug) and brivaracetam (third generation anti epileptic drug) are promising examples of prescribed antiepileptic racetams that act on SV2A. Brivaracetam is a newer antiseizure medication than levetiracetam. It has a more selective action on the synaptic vesicle glycoprotein 2A binding site, and it seems to provide a more favorable neuropsychiatric profile. Bioanalytical methods are widely used for quantitative estimation of drugs and their metabolites in biological matrices. Various analytical methods such as spectrophotometry, HPLC, GC, TLC, qNMR and UPLC have been used in laboratories for the quantitative analysis of antiepileptic racetams in biological samples throughout all phases of clinical research and quality control. The article incorporates various reported methods developed to help analyst in choosing crucial parameters for new method development of antiepileptic racetams and its derivatives and also enumerates metabolites, impurities reported so far.

Key words: Levetiracetam, Brivaracetam, Racetams, Antiepileptic, Analytical review.

## *1- Introduction*

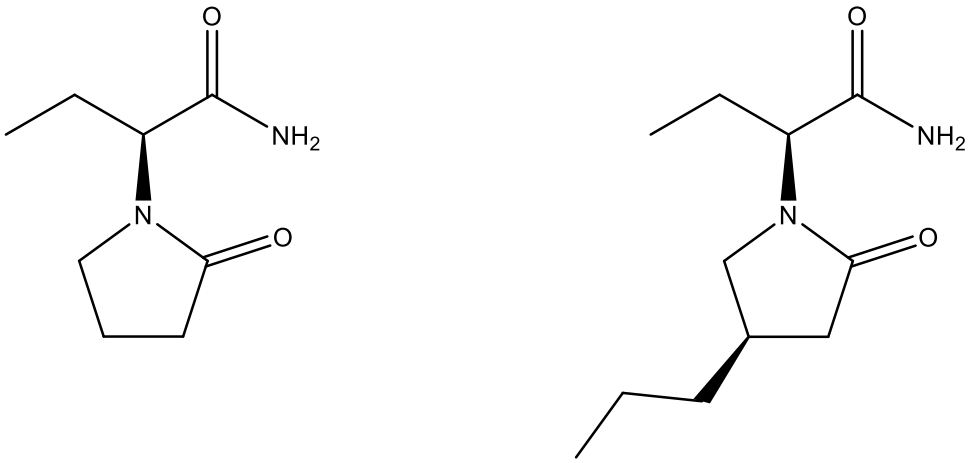
There has been a significant advance in the determination of anticonvulsive medications during the past 20 years. Widespread applications of bioanalytical methods include the evaluation and interpretation of bioequivalence, pharmacokinetic, and toxicological research, as well as the quantitative measurement of medicines in physiological matrices.

Chromatographic methods such as gas chromatography (GC), liquid chromatography mass spectrometry (LCMS), high performance liquid chromatography (HPLC) are frequently employed in labs for the quantitative analysis of biological samples and drug compounds during all stages of a drug development for research and quality control.

The present review covers the wide range of analytical techniques used in determination of levetiracetm and brivaracetam either alone or with other medications in their pharmaceuticals and biological matrices. It also incorporates records for simultaneous estimations with other drug members, impurities and metabolites.

Levetiracetam, (S)- α- ethyl- 2 oxo- 1- pyrrolidine acetamide, and brivaracetam (2S)-2-[(4R)-2-oxo-4-propyltetrahydro-1H-pyrrol-1-yl] butanamide, (figure 1) (Patsalos & St Louis, 2018) are promising examples of racetams with approved anti-epileptic activity. Levetiracetam binds to the synaptic vesicle protein SV2A, which is involved in synaptic vesicle exocytosis of glutamate, causing neurotransmitter release to be suppressed (Lynch et al., 2004). Negative modulators of GABA- and glycine-gated currents are inhibited also by levetiracetam, and N-type calcium currents are partially inhibited (Rigo et al., 2002).

Brivaracetam is 10- to 30-fold more potent than levetiracetam due to its high lipid solubility and rapid brain penetration. It binds with high and selective affinity to synaptic vesicle protein SV2A, which is involved in synaptic vesicle exocytosis. Brivaracetam is a partial antagonist of sodium channels, leading to blockade of voltage-dependent excitatory currents (Patsalos & St Louis, 2018). In addition, it inhibits calcium dependent exocytosis of synaptic vesicles with excitatory neurotransmitters and so decreases its release.



Levetiracetam Brivaracetam

**Figure 1. Chemical Structure for Levetiracetam and Brivaracetam**

Levetiracetam (LEV) is a white to off - white crystalline powder. It is very soluble in water (104.0 g/100 mL). Levetiracetam has a chiral center and is administered as the S-enantiomer, which possesses higher biological activity than the racemate. Log p = -0.65 and pKa value of 15.74 (Petruševska et al., 2015).

Brivaracetam is a white to off-white non-hygroscopic crystalline powder, highly soluble in water and also in polar organic solvents ; log P 0.66 and pKa values are 16.29 and -0.57 (Bigus, Tsakovski, Simeonov, Namieśnik, & Tobiszewski, 2016; Wishart et al., 2018).

### *2- Analytical review of Levetiracetam*

Liquid chromatographic methods for assay of LEV were reported in BP using 2-propanol and heptane (18:82, v/v ) as mobile phase (Commission:) and United States Pharmacopeia using solution A: Acetonitrile and Buffer (1:19), solution B: Acetonitrile at gradient elution mode (US Pharmacopeial Convention). Levetiracetam detected at 205 nm for both methods. Several analytical methods for determination of LEV were published including the following.

**Spectrophotometric and spectrofluorometric methods**

LEV was quantified in tablet dosage forms using three spectrophotometric methods, which were based on the formation of chloroform extractable complex of LEV with Bromocresol green, Bromophenol blue and Bromothymol blue (Srinivasu, Rao, Raju, & Prakash, 2008). The maximum UV absorbance was measured at 435 nm, 454 nm and 415 nm for the three formed complexes, respectively. LEV was determined in pharmaceuticals using visible spectrophotometric method based on charge transfer complex formation of the drug with chloranilic acid. The resulted pink complex was measured at 530 nm (Bhaskararao et al., 2010).

Ganapathy *et al*. (Ganapathy, Raju, Sankar, & Naidu, 2010) developed different three UV-visible methods for LEV assay in pharmaceuticals. The first method was based on the formation of colored compound as a result of LEV reaction with 3-Methyl-2-benzo thiazolinone hydrazone (MBTH) and cerric ammonium sulphate. Second method was based on the formation of colored species on treatment of LEV with $FeCl_3$ and 1, 10 phenanthroline. The third one was based on UV Spectrophotometric determination of LEV in methanol at 210 nm.

Colorimetric determinations for assessment of LEV in its pharmaceuticals were developed. The first method was based on the charge transfer complexation reaction between LEV and p-chloranilic acid to form a violet chromogen detected at 440 nm. The second method is based on the reaction of LEV with ferric chloride and potassium ferricyanide to form a green colored compound with absorption maxima at 750 nm (Indupriya, Chandan, Gurupadayya, & Sowjanya, 2011).

LEV also was determined using flow injection analysis in presence of topiramate and piracetam in pharmaceuticals (Hadad, Abdel-Salam, & Emara, 2011). The method is based on the reaction with ortho-phtalaldehyde and 2-mercaptoethanol in a basic buffer and measurement of absorbance at 295 nm.

Levetiracetam was determined spectrofluorimetrically in pharmaceutical tablets and human plasma by derivatization using 4-Chloro-7-nitrobenzofuran-2-oxo-1,3-diazole (NBD-Cl) and measured at $\lambda_{em}/_{ex}$ of 551/465 nm. While a binary mixture of LEV and topiramate were determined by the fourth derivative synchronous fluorescence measurement after their reaction with NBD-Cl. In this method, the fourth derivative synchronous spectra were estimated as peak to peak measurement at 493–497 and 490.5–495 nm corresponding with zero-contribution of LEV and topiramate, respectively (El-Yazbi, Wagih, Ibrahim, & Barary, 2016).

Kumar *et al.* (Kumar, Rao, Bharath, & Avinash, 2017) developed another spectrophotometric method for determination of LEV in pharmaceutical formulation based on its reaction with ammonium molybdate solution and hydrazine sulphate solution to produce intense blue colored molybdenum blue complex. The produced complex showed a λmax at 823nm.

LEV was determined in its pharmaceutical formulation after condensation of its amino group with acetylacetone and formaldehyde according to Hantzsch reaction. The product is a highly fluorescent yellow colored dihydropyridine derivative that was measured at 390 nm (Ibrahim, El-Yazbi, Wagih, & Barary, 2017).

Chakure *et al.* (Chakure, Gholve, & Kumar, 2018) reported a spectrophotometric method for determination of LEV in tablets and stress degradation study, where the drug showed maximum absorption at 265.0 nm in distilled water.

***Quantitative Nuclear Magnetic Resonance (qNMR)***

Proton nuclear magnetic resonance spectroscopy practice ($^1$H NMR) was applied to quantify LEV in either pure or pharmaceutically available tablets by scanning different experimental settings applying DOE as a statistical methodology (N. M. Mansour, D. T. El-Sherbiny, F. A. Ibrahim, & H. I. El-Subbagh, 2022b).

***Chromatographic Methods.***

**a- Thin Layer Chromatography (TLC).**

High performance thin layer chromatography (HPTLC) was applied for determination of LEV simultaneously with lamotrigine and zonisamide in human plasma. Chromatographic separation was achieved on silical gel 60F254 plates using ethylacetate, methanol, and ammonia (91:10:15, v/v/v) as developing system. Quantitative analysis was carried out by densitometry at a wavelength of 312, 240 and 210 nm for lamotrigene, zonisamide and LEV, respectively (Antonilli, Brusadin, Filipponi, Guglielmi, & Nencini, 2011).

**b- High Performance Liquid Chromatography (HPLC).**

Various HPLC methods were applied for determination of LEV can summarized as follow (Table 1):

**c- Ultra-Performance Liquid Chromatography (UPLC)**

Ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was developed for the determination of LEV in plasma of neonates (Blonk, van der Nagel, Smit, & Mathot, 2010) . LEV was separated on an Acquity UPLC BEH C18 column using gradient mode and mobile phase consisted of solution A (0.1% formic acid and10 mM ammonium formate in water pH 3.5) and solution B (0.1% formic acid in methanol) with a flow rate of 0.4 ml/min. LEV was detected using positive ion electrospray ionization followed by tandem mass spectrometry (ESI-MS/MS).

Olah *et al*. (Olah, Bacsoi, Fekete, & Sharma, 2012) reported an ultra-high-performance liquid chromatography–photodiode absorbance method for analysis of LEV in plasma samples of patient with epilepsy. The column for separation is BEH C18 column and a mixture of acetonitrile, 0.01 M phosphate buffer (pH 5-6.6) (10/90 v/v) was the mobile phase.

**Table 1. Summary of Reported HPLC Methods for Determination of Levetiracetam.**

| Matrix | Column | Mobile Phase | Detection | Ref. |
|---|---|---|---|---|
| LEV in human plasma | C8 | Methanol, acetonitrile and 3 mM phosphate buffer, containing 0.5 mL triethylamine (6:5:89, v/v/v) at pH of 6.0. | diode array detection at 205 nm | (Pucci et al., 2004) |
| LEV in human plasma | porous graphitic carbon | Diluted phosphoric acid/acetonitrile (gradient mode) | UV detection at 205 nm | (Martens-Lobenhoffer & Bode-Böger, 2005) |
| LEV simultaneously with zonithamide in plasma/serum | Phenyl Hexyl column | 1 L deionized water, 50 mL of dibutylammonium phosphate, and 100 rnL of methanol. | UV detection at 220 nm | (Juenke, Brown, Urry, & McMillin, 2006) |
| LEV in human plasma | C18 | Ammonium acetate buffer (pH 3.2), acetonitrile in a ratio of (20:80, v/v) | MS/MS | (Jain, Subbaiah, Sanyal, Pal, & Shrivastav, 2006) |
| LEV in plasma, serum, saliva | C18 | (A): a mixture of 15 mmol/L ammonium acetate and methanol (98:2, v/v), 0.1% acetic acid, (B): a mixture of 15 mmol/L ammonium acetate and methanol (3:97, v/v), 0.1% acetic acid (gradient mode) | MS/MS | (Guo, Oswald, Mendu, & Soldin, 2007) |
| LEV in human plasma | C18 | Methanol: ammonium buffer, 10 mmol/L pH 10 (30:70, v: v) | diode array detection at 200 nm | (Lancelin et al., 2007) |
| LEV in tablet dosage form | C18 | Acetonitrile and 0.03 M potassium dihydrogen phosphate (pH 3.0) (15:85 v/v), flow rate 1 mL /min | UV detection at 210 nm | (Raju, Rao, Prakash, Mukkanti, & Srinivasu, 2008) |
| LEV in human plasma | C18 | Methanol, water, formic acid (97:3:0.25, v/v/v), flow rate 0.2 ml/min | MS/MS | (Matar, 2008) |
| LEV in plasma of patients with epilepsy | Hydro-RP | Potassium dihydrogen phosphate buffer (50 mM, pH 4.5) and acetonitrile (94:6, v/v), flow rate 1.5 mL/min | UV detection at 205 nm | (Contin, Mohamed, Albani, Riva, & Baruzzi, 2008) |
| LEV in pharmaceutical formulation | C18 | 0.05 M Potassium dihydrogen phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and methanol in the ratio 70:30 v/v. flow rate 1.2 mL/min | UV detection at 210 nm | (Rao, 2010) |
| LEV in human plasma and serum | C18 | (A) 0.1% formic acid and (B) methanol at pH of 2.5 (gradient mode) | MS/MS | (Mendu & Soldin, 2010) |
| Application to pharmacokinetic studies | Hydro-RP | Potassium dihydrogen phosphate buffer (50 mM, pH 4.5) and acetonitrile (94:6, v/v) | diode array detection at 205 nm | (Zufia, Aldaz, Ibanez, Giraldez, & Viteri, 2010) |
| LEV in plasma | C18 | 0.1% Formic acid in water and acetonitrile (40:60 v/v) | MS/MS | (Yeap & Lo, 2014) |

| Matrix | Column | Mobile Phase | Detection | Ref. |
|---|---|---|---|---|
| enantioselective analysis of LEV in a pharmaceutical formulation | chiral-α1-acid glycoprotein column | Phosphate buffer (pH = 7), flow rate 0.7 mL/min | UV detection at 210 nm | (Heydari & Shamsipur, 2015) |
| LEV in plasma and serum of patients with epilepsy | C18 | 50 mM Potassium dihydrogen phosphate–acetonitrile (90:10, v/v) at a pH of 5.5, flow rate 1.0 mL/min | UV detection at 205 nm | (Engelbrecht, Grobler, & Rheeders, 2017) |
| LEV simultaneously with zonisamide, lamotrigine, pentylenetetrazole, and pilocarpine in rat plasma and brain matrices. | C18 | Acetonitrile (7.5%) and a mixture (92.5%) of water-triethylamine (99.5:0.05, v/v, pH 6.4), flow rate 1 mL min | UV detection at 205 nm | (Fonseca, Rodrigues, & Alves, 2018) |
| LEV in blood spot | C18 | (A) 10 mmol/L ammonium formate with 0.15 % formic acid, (B) methanol, (gradient mode). | MS/MS | (Linder, Hansson, Sadek, Gustafsson, & Pohanka, 2018) |
| LEV simultaneously with lacosamide and zonisamide in human plasma | C18 | Water and acetonitrile (gradient mode) | diode array detection at 220 nm | (Goncalves, Alves, Bicker, Falcao, & Fortuna, 2018) |
| LEV in plasma of neonates | C18 | 0.1% Formic acid in 10.0 mM ammonium acetate and methanol in a ratio of (10: 90, v/v), | MS/MS | (Jenjirattithigarn et al., 2018) |
| LEV simultaneously with pyridoxine HCl in prepared tablets | C8 | Methanol and 25 mM $KH_2PO_4$ buffer pH 3 (38.4:61.6, v/v), flow rate 0.8 mL/min | UV detection at 214 nm | (Hashem & El-Sayed, 2018) |
| LEV in plasma | Hydro-RP | 50 mM potassium dihydrogen phosphate and acetonitrile (gradient mode) | UV detection at 195 nm | (Kalaria, Dahmane, Armahizer, McCarthy, & Gopalakrishnan, 2018) |
| LEV in oral solution and application to chemical kinetics | C18 | Methanol : water (30:70 %, v/v), flow rate of 1 mL/min | UV detection at 205 nm | (Sonawane, Chhajed, Jadhav, Thombre, & Kshirsagar, 2020) |
| LEV simultaneously with piracetam and brivaracetam in pharmaceuticals, human plasma and *in-vitro* dissolution testing | C 18 | Methanol : Acetonitril: water (10 : 20 : 70, v/v/v) | UV detection at 210 nm | (Mansour, El-Sherbiny, Ibrahim, & El Subbagh, 2022) |

LEV was also determined simultaneously with gabapentin, lamotrigine, monohydroxy Derivative (MHD) of oxcarbazepine, and zonisamide in serum (Palte et al., 2018). The method was based on simple protein precipitation with acetonitrile containing isotopically labeled internal standard. Quantification of analytes was accomplished using electrospray ionization in positive ion mode and collision induced dissociation MS.

Joetta *et al*. (Juenke, Brown, Johnson-Davis, & McMillin, 2011) developed a method for determination of LEV simultaneously with gabapentin in plasma for simultaneous drug monitoring. This method used an Acquity UPLC HSS T3, (Waters) column and the elution occurs using a linear gradient of acetonitrile and water, each having 0.1% formic acid.

**d- Gas Chromatography (GC)**

Gas chromatographic mass spectrometric (GC/MS) technique was also applied for the determination of LEV and its enantiomer (R)-α-ethyl-2-oxo-pyrrolidine acetamide in dog plasma and urine. Separation of the enantiomers were achieved on a chiral cyclodextrin capillary column and detected using ion trap mass spectrometry. The fragmentation pattern of the enantiomers was further investigated using tandem mass spectrometry (Isoherranen et al., 2000).

Another GC method was applied for determination of LEV in human plasma after dispersive liquid-liquid microextraction. The method was based on using a linear temperature program, capillary fused silica column, and helium as the carrier gas (Alcantara et al., 2016). Alvarez *et al*. (Alvarez-Freire, Rodríguez, Barrera, Cabarcos-Fernandez, & Tabernero-Duque, 2021) developed GC/MS method for the determination of LEV in human plasma, the determination was performed in selected ion monitoring mode using deuterated levetiracetam as internal standard.

***3- Analytical review of Brivaracetam***

Few analytical methods were published for the determination of BRV. A brief description of the reported methods for BRV can be summarized as follows:

***Spectrophotometric analysis***

Brivaracetam was assayed with LEV and piracetam in their ternary mixture and dosage forms using double devisor ratio spectra derivative method where measurements were taken at 229.7 nm, Corresponding to a maximum wavelength (N. M. Mansour, D. El-Sherbiny, F. A. Ibrahim, & H. El-Subbagh, 2022a).

***Chromatographic Methods.***

**a- High-Performance Liquid Chromatography (HPLC):**

HPLC coupled with UV detection method was reported for the resolution of brivaracetam stereoisomeric impurities. Resolution was performed using a chiral column and mobile phase consisted of 10 mM ammonium bicarbonate and acetonitrile in a ratio of (65:35, v/v). The eluents were detected at 215 nm (Baksam, Pocha, Chakka, Ummadi, & Kumar, 2020).

Another HPLC coupled with ultra violet detection method was also developed for determination of BRV simultaneously with piracetam and carbamazepine in their pharmaceuticals and human plasma (Mansour, El-Sherbiny, Ibrahim, & El Subbagh, 2021). The method applied C 18 column as a stationary phase and a mixture of acetonitrile and water in a ratio of 30 : 70, v/v as a mobile phase. The eluents were detected at 215 nm.

**b- Ultra-Performance Liquid Chromatography (UPLC):**

Ultra-Performance Liquid Chromatography/tandem mass spectrometry method (UPLC/MS/MS) for identification and quantification of BRV in plasma was developed. Separation was achieved with C18 column and mobile phase combination of acetonitrile and 0.1% formic acid in water (Iqbal, Ezzeldin, & Al-Rashood, 2017).

Vishweshwar *et al* (Vishweshwar, Babu, & Muralikrishna, 2018) developed an Ultra-Performance Liquid Chromatography stability indicating method for determination of BRV, its related impurities and degradation products. The sample was eluted on a C18 column and the mobile phase contained water/ acetonitrile (gradient mode). The eluted compounds were estimated at 230 nm using photo diode array detector.

Iqbal *et al* (Iqbal, Khalil, Ezzeldin, & Al-Rashood, 2018) developed an Ultra-Performance Liquid Chromatography/tandem mass spectrometry method (UPLC/MS/MS) for determination of BRV simultaneously with suvorexant and lorcaserin in human plasma. Separation was performed using C18 column and 10mM ammonium acetate/acetonitrile/formic acid in a ratio of (15:85:0.1%; v/v/v) as the mobile phase. Multiple reaction monitoring transitions were selected for the detection and quantification of each compound.

***Quantitative Nuclear Magnetic Resonance (qNMR)***

Proton nuclear magnetic resonance spectroscopy practice ($^1$H NMR) was applied to quantify BRV in either pure or laboratory prepared tablets by scanning different experimental settings applying DOE as a statistical methodology (Mansour, El-Sherbiny, et al., 2022b).

## *4- Conclusion*

The present review incorporates outline of various methods and techniques used in quantifying levetiracetam and brivaracetam using reports of analytical studies. Different analytical methods have been applied utilizing factors such instruments, stationary phase types, analytical columns, and mobile phases.

The review would assist the analytical chemist in understanding the essential solvents and their combinations for his set of instruments that are available in the analytical laboratory. With the proper comparisons provided in the published records, analytical chemists can learn about the advantages of one technique over another. In addition to single drug profile records, review also includes records of comparative investigations of one or more compounds belonging to the same class.

The most efficient set of parameters should lower the cost of the study and shorten the time needed to produce a trustworthy analytical procedure.

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