A Review on Analytical Methods– Levetiracetam

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ABSTRACT: Levetiracetam is a pyrrolidone-family antiepileptic drug of the second generation with a broad spectrum of activity. The US Food and Drug Administration authorized Levetiracetam as a broad-spectrum antiepileptic drug in 1999. The hydrophilic groups of Levetiracetam differentiate it from other earlier antiepileptic drugs. The following review covers the analytical techniques for the analysis of Levetiracetam.

KEYWORDS: Levetiracetam (LVT); Antiepileptic drug; analytical technique; spectrophotometry; HPLC, LC-MS.

I. INTRODUCTION:
The U.S. Food and Drug Administration has authorised levetiracetam (Keppra), a novel antiepileptic drug, as an adjunctive therapy for partial myoclonic and tonic-clonic seizures as well as a monotherapy treatment for epilepsy in conditions of partial seizures. Other mental and neurological problems such as Tourette syndrome, autism, and anxiety disorders may be helped by levetiracetam. LVT has a linear pharmacokinetic profile and is nearly completely absorbed after oral administration. Chemically it is (2S)-2-(2-oxopyrrolidin-1-yl)butanamide (figure 1). It has a molecular formula C₈H₁₄N₂O₂ (MW 170.20). Levetiracetam is very soluble in water and methanol, and chloroform. Levetiracetam is practically insoluble in n-hexane. LVT has a linear pharmacokinetic profile and is nearly completely absorbed after oral administration. It is less than 10% protein bound. LVT has no effect on or is influenced by the cytochrome P450 system. It is mostly eliminated via the kidneys. In renal failure, its plasma half-life can be extended. This is a structural analog of piracetam that binds to the synaptic vesicle protein SV2A, obstructing nerve transmission across synapses.

Fig.1 Structure of Levetiracetam
The present review article summarises the analytical techniques so far developed, such as spectrophotometry\(^4\)\(^-\)\(^10\) (Table 1), high performance thin layer chromatography\(^1\)\(^1\) (Table 2), high-performance liquid chromatography\(^1\)\(^2\)\(^-\)\(^26\) (Table 3), liquid chromatography-mass spectrometric methods\(^2\)\(^7\)\(^-\)\(^32\) (Table 4) for the determination of Levetiracetam (LVT) and some of the analytical parameters were highlighted.

1. **Spectrophotometric method:**
   Table 1. Determination of Levetiracetam by UV Spectrophotometric Method:
<table>
<thead>
<tr>
<th>Solvent/Reagent</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>Linearity (µg/ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chloro phenyl hydrazine and 2 ml (0.25 %) Anthranilic acid</td>
<td>560</td>
<td>-</td>
<td>[4]</td>
</tr>
<tr>
<td>Water</td>
<td>209</td>
<td>2-10</td>
<td>[5], [6]</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>221</td>
<td>30-90</td>
<td>[7]</td>
</tr>
<tr>
<td>2,4- Dinitrophenylhydrazine</td>
<td>455</td>
<td>30-130</td>
<td>[8]</td>
</tr>
<tr>
<td>Methanol</td>
<td>220</td>
<td>10-50</td>
<td>[9]</td>
</tr>
<tr>
<td>Distilled water</td>
<td>265</td>
<td>2-12</td>
<td>[10]</td>
</tr>
</tbody>
</table>

2. **High Performance Thin Layer Chromatography (HPTLC):**
   Table 2. Determination of Levetiracetam by HPTLC Method:
<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>(\lambda) (nm)</th>
<th>Stationary Phase</th>
<th>Linearity Range (ng/band)</th>
<th>LOD (ng/band)</th>
<th>LOQ (ng/band)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene: Acetone: Methanol(6:2:2) v/v/</td>
<td>210</td>
<td>Silica gel 60 F(_{254})</td>
<td>500-3000</td>
<td>19.76</td>
<td>65.89</td>
<td>[11]</td>
</tr>
</tbody>
</table>

3. **High Performance Liquid Chromatography (HPLC):**
   Table 3. Determination of Levetiracetam by HPLC Method:
<table>
<thead>
<tr>
<th>Technique</th>
<th>Mobile Phase</th>
<th>Flow rate</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP-HPLC</td>
<td>Methanol: Water:TEA 75:25:05 (V/V)</td>
<td>1.0 ml/min</td>
<td>0.05</td>
<td>0.15</td>
<td>[12]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>mixture of 0.1 g/L of triethylamine and acetonitrile(70: 30 v/v)</td>
<td>1.0 ml/min</td>
<td>1.66</td>
<td>8.7</td>
<td>[13]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>MeOH and 25mM KH(_2)PO(_4) buffer pH 3 (38.4:61.6, v/v)</td>
<td>0.8 ml/min</td>
<td>0.20</td>
<td>1.56</td>
<td>[14]</td>
</tr>
<tr>
<td>RP-HPLC in Human serum</td>
<td>ammonium acetate buffer (10mM, pH 5) and acetonitrile (50:50v/v)</td>
<td>0.3 ml/min</td>
<td>0.8</td>
<td>2.5</td>
<td>[15]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>diphasic sodiumphosphate buffer and acetonitrile in a ratio of 80:20</td>
<td>1.5 ml/min</td>
<td>0.6-1.2</td>
<td>4</td>
<td>[16]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>buffer solution (pH 2.8) and acetonitrile in the ratio of 90:10</td>
<td>1.2 ml/min</td>
<td>-</td>
<td>-</td>
<td>[17]</td>
</tr>
<tr>
<td>HPLC</td>
<td>Methanol: ACN: Water (60:20:20) pH3</td>
<td>1 ml/min</td>
<td>0.0719</td>
<td>0.218</td>
<td>[9]</td>
</tr>
<tr>
<td>HPLC</td>
<td>1 L deionized water, one vial of Waters (Milford, MA) D4 mobile phase</td>
<td>0.5 ml/min</td>
<td>-</td>
<td>1</td>
<td>[18]</td>
</tr>
<tr>
<td>Matrices</td>
<td>Mobile Phase</td>
<td>Column</td>
<td>Method</td>
<td>Flow Rate</td>
<td>LOQ (ng/ml)</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>------------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Human plasma</td>
<td>a mixture of 0.1% formic acid in water and ACN (40:60 v/v)</td>
<td>LC-MS Agilent Zorbax SB-C18</td>
<td>isocratic elution</td>
<td>0.5 ml/min</td>
<td>0.5</td>
</tr>
<tr>
<td>Human plasma</td>
<td>30 mM aq. Orthophosphoric acidsolution/methanol(70:30) (A) and 10 mM aq. ortho-phosphoricacid solution</td>
<td>LC GL Sciences IntersilODS-3 (A) and Restek Ultra PFPP column</td>
<td>Gradient elution</td>
<td>1 ml/min</td>
<td>6</td>
</tr>
</tbody>
</table>
Human plasma/serum/saliva

97% methanol in 15 mmol/1 ammonium acetate (v/v), 0.1% acetic acid

LC-MS/MS C-18 column

isocratic elution

1 ml/min

- [29]

Human plasma

5 mM Ammonium acetate (adjusted to pH 3.2 with Glacial acetic acid): Acetonitrile (20:80) Clonazepam (Internal standard)

LC-MS/MS Electron Betasil C-18

isocratic elution

0.5 ml/min

0.125 [30]

Human plasma

0.1% formic acid in 10.0 mM ammonium acetate (A) and 100% methanol (B)

LC-MS/MS C18 SPE

isocratic elution

0.4 ml/min

0.50 [31]

Human plasma

0.1% formic acid–10mM Mammnonium formate in water (pH 3.5) (mobile phase solution A) and 0.1% formic acid in methanol (mobilephase solution B)

UPLC–MS/MS

Gradient elution

0.4 ml/min

0.5 [32]

II. CONCLUSION:

For the estimation of levetiracetam in bulk, pharmaceutical formulations, and biological samples, various analytical methods, including UV, HPLC, HPTLC, and hyphenated techniques such as LC-MS, UPLC-MS/MS methods, were described. This review article will help readers understand the analytical techniques described for quantifying Levetiracetam.

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