CHAPTER-V

Application of UHPLC for determination of Gabapentin drug substance and its related impurities
**Introduction:**

In the last twenty years, 14 new Anti Epileptic Drugs (AEDs) have entered the market in the United States and/or Europe[^1][^2]. These drugs are Eslicarbazepine acetate, Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Oxcarbazepine, Pregabalin, Rufinamide, Stiripentol, Tiagabine, Topiramate, vigabatrin and Zonisamide. Eslicarbazepine acetate, lacosamide, rufinamide, and stiripentol are not yet approved in the United States. The development of organic chemistry has provided the basis for synthesis of anticonvulsants or antiepileptic drugs of increasing potency and selectivity.

The newer AEDs are sometimes characterized as second or third generation drugs. In comparison to the older AEDs, the newer agents often have wider therapeutic ranges and fewer serious adverse effects. Like some of the older AEDs, the newer agents may also be used for other conditions such as bipolar disorder ('manic depression'), chronic pain syndromes (e.g., fibromyalgia, trigeminal neuralgia), or migraine headaches[^1][^3]. In general, the clinical utility of therapeutic drug monitoring has not been established in clinical trials for these new anticonvulsants, and clear guidelines for drug monitoring have yet to be defined.

**Pain:**

Gabapentin provides significant pain relief in about a third of people who take it for fibromyalgia or chronic neuropathic pain[^4]. It is also effective in reducing narcotic usage post operatively[^5] and is helpful in neuropathic pain due to cancer[^6]. It has not been shown to be useful for HIV associated sensory neuropathy[^7]. When used for neuropathic pain it does not appear superior to Carbamazepine[^8]. Further evidence is needed to determine if it is effective for migraine prevention[^9]. It appears to be as effective as Pregabalin and costs less[^10]. It does not appear to be of benefit in treating complex regional pain syndrome[^11]. Some literature has suggested that it may be used as a broad spectrum analgesic[^12].

**Background on Therapeutic Drug Monitoring of Antiepileptic Medications:**

Drugs used to prevent and treat seizures AEDs have been among the most common medications for which therapeutic drug monitoring (TDM) is performed[^13][^14].
Traditionally, TDM has been applied mainly to the 'older' or first-generation AEDs that have been on the market in the United States and Europe for several decades, namely carbamazepine, phenobarbital, phenytoin, primidone, and valproic acid. These first-generation AEDs in general have narrow therapeutic ranges and significant inter-individual variability in their pharmacokinetics (absorption, distribution, metabolism, and excretion). Only two randomized, controlled studies of AED TDM have been conducted and neither showed clinical benefits. Both studies did show, however, that physicians often apply information from TDM incorrectly, diminishing the clinical impact of TDM [15, 16]. Better education of medical practitioners on TDM is a priority for the future.

TDM of AEDs faces three main challenges [2]. Firstly, seizures occur irregularly, sometimes with long periods of time between episodes. Consequently, long-term observation of any therapy for seizures may be needed to assess clinical benefit. Secondly, some AEDs produce adverse effects that may be difficult to distinguish from the underlying neurologic disease. Lastly, there are no simple laboratory tests or diagnostic procedures that can assess the clinical efficacy of AEDs. Clinical observation and relatively labor-intensive procedures such as the electroencephalogram (EEG) remain the mainstays of clinical assessment. For example, one of the study conducted by P Crawford et.al., from the department of Neurology, Walton Hospital, Liverpool UK [17] for Gabapentin as an antiepileptic drug in man; Gabapentin, l-(aminomethyl)cyclohexane acetic acid, is a GABA analogue whose antiepileptic properties were tested in a double blind cross-over trial design as add-on therapy in a dose ranging study which compared 300 mg, 600 mg and 900 mg/day (each dose given for 2 months) in 25 patients with severe partial and generalized epilepsies. A dose related antiepileptic effect was observed. All three doses were well tolerated and no psychometric impairment was noted. No significant drug interactions were seen. The drug appears worthy for further assessment.

Reasons for Applying TDM to AEDs:

There are multiple reasons why TDM may be useful in the clinical management of AED therapy. A common reason is that the pharmacokinetics of the drug shows significant inter-individual variability [18-20]. If the pharmacokinetics is very consistent
and predictable, then dosing of the drug can often be done without TDM. Metabolism (biotransformation) is a major pharmacokinetic factor that can affect AEDs. Variability in metabolism may be due to impaired organ function (typically kidney or liver), genetic factors (pharmacogenetics) or drug-drug or drug-food interactions. Several AEDs, namely carbamazepine, phenobarbital and phenytoin, are well-known 'inducers' (stimulators) of 'drug-metabolizing' enzymes in the liver and other organs.\(^{[21]}\)

AEDs are often used in patients with some degree of renal impairment. Renal insufficiency can alter AED pharmacokinetics by decreased clearance of drug and/or metabolites, or by removal of drug during dialysis procedures. For some AEDs, there has been little investigation of the effect of dialysis on AED plasma concentration. In general, AEDs with low degrees of plasma protein binding are cleared more effectively by dialysis than those AEDs with high degrees of protein binding.\(^{[22]}\) AEDs that show variable and unpredictable pharmacokinetics are good candidates for TDM.\(^{[14]}\) For some medications that are highly (>90%) bound to serum proteins, monitoring of free (unbound) drug concentrations may be clinically useful.\(^{[23]}\) A number of factors may alter serum protein concentrations including liver disease, old age, and pregnancy. Free drug concentrations are typically measured by analyzing the concentration of drug present in an ultrafiltrate of plasma or serum. The technical challenge is that free drug concentrations for drugs that are highly protein bound are substantially lower than total drug concentrations. Some analytical methods that may be suitable for measuring total drug concentrations may have insufficient analytical sensitivity to accurately measure the full range of clinically useful free drug concentrations.\(^{[23]}\) In addition, the ultrafiltration process is not easily automated and thus adds manual processing time to the clinical laboratory analysis of AEDs that require determination of free drug concentration.

The Challenge behind Establishing Reference Ranges for AEDs:

Reference ranges for the newer AEDs have generally been difficult to establish.\(^{[14]}\) These drugs are usually effective over a wide range of serum/plasma concentrations but with substantial inter-individual variation in response. Ideally, TDM would guide physicians towards serum/plasma concentrations that optimize seizure control, while avoiding or at least minimizing toxic effects. The 'reference range' of an AED can be
defined by two limits—a lower limit below which therapeutic effect is unlikely and an upper limit above which toxicity is likely.\textsuperscript{[14]}

In addition, reference ranges may vary with different types of seizures, or when AEDs are used to treat other clinical conditions since a population that may respond quite differently from patients with more easily treatable disease.

TDM has been applied periodically to determine whether the concentration is staying near the individual therapeutic concentration.\textsuperscript{[24, 25]} Drug monitoring can be especially important when changes in the patient occur that alter AED pharmacokinetics, e.g., pregnancy, impaired kidney or liver function, or concomitant therapy with enzyme-inducing or inhibiting drugs. Table 1 summarizes the pharmacokinetic properties of the newer AEDs.

\textbf{Table 1: Pharmacokinetic properties and reference ranges for the AEDs.}[\textsuperscript{[14]}]

<table>
<thead>
<tr>
<th>Drug</th>
<th>OB (%)</th>
<th>SPB (%)</th>
<th>TPC (h)</th>
<th>HACEI a</th>
<th>HPCEI a</th>
<th>RRS (mg/L) f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eslicarbazepine acetate</td>
<td>&gt;80</td>
<td>30</td>
<td>1–4</td>
<td>20–24</td>
<td>20–24</td>
<td>Not established</td>
</tr>
<tr>
<td>Felbamate</td>
<td>&gt;90</td>
<td>25</td>
<td>2–6</td>
<td>16–22</td>
<td>10–18</td>
<td>30–60</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>&lt;60</td>
<td>0</td>
<td>2–3</td>
<td>5–9</td>
<td>5–9</td>
<td>2–20</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>≥95</td>
<td>15</td>
<td>0.5–4</td>
<td>12–13</td>
<td>12–13</td>
<td>5–10</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>≥95</td>
<td>55</td>
<td>1–3</td>
<td>15–35b</td>
<td>8–20</td>
<td>3–14</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>≥95</td>
<td>0</td>
<td>1</td>
<td>6–8</td>
<td>6–8</td>
<td>12–46</td>
</tr>
<tr>
<td>Oxcarbazepine \textsuperscript{e}</td>
<td>90</td>
<td>40</td>
<td>3–6</td>
<td>8–15</td>
<td>7–12</td>
<td>3–35</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>≥90</td>
<td>0</td>
<td>1–2</td>
<td>5–7</td>
<td>5–7</td>
<td>2.8–8.3</td>
</tr>
<tr>
<td>Rufinamide</td>
<td>85</td>
<td>30</td>
<td>5–6</td>
<td>8–12</td>
<td>≤8</td>
<td>Not established</td>
</tr>
<tr>
<td>Stiripentol \textsuperscript{d}</td>
<td>≥90</td>
<td>99</td>
<td>1–2</td>
<td>Variable \textsuperscript{e}</td>
<td>Variable \textsuperscript{e}</td>
<td>4–22</td>
</tr>
<tr>
<td>Tiagabine \textsuperscript{d}</td>
<td>≥90</td>
<td>96</td>
<td>1–2</td>
<td>5–9</td>
<td>2–4</td>
<td>0.02–0.2</td>
</tr>
<tr>
<td>Topiramate</td>
<td>≥80</td>
<td>15</td>
<td>2–4</td>
<td>20–30</td>
<td>10–15</td>
<td>5–20</td>
</tr>
<tr>
<td>Vigabatrin</td>
<td>≥60</td>
<td>0</td>
<td>1–2</td>
<td>5–8</td>
<td>5–8</td>
<td>0.8–36</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>≥65</td>
<td>50</td>
<td>2–5</td>
<td>50–70</td>
<td>25–35</td>
<td>10–40</td>
</tr>
</tbody>
</table>
Legends: OB-Oral Bioavailability; SPB- Serum protein binding; TPC- Time to peak concentration; HACEI-Half-life in Absence of Concomitant Enzyme Inducers; HPCEI-Half-life in Presence of Concomitant Enzyme Inducers and RRS- Reference Range in Serum a Enzyme inducers include carbamazepine, phenobarbital, phenytoin, rifampicin, and St. John's wort. References for drugs whose half-lives are altered in patients receiving liver enzyme inducers: felbamate [26], lamotrigine [27], oxcarbazepine [28], rufinamide [29], tiagabine [30], topiramate [31] and zonisamide [20]. Half-life increases to 30–90 hr during concomitant therapy with valproic acid (enzyme inhibitor). All parameters refer to the active metabolite 10-hydroxycarbazepine. Monitoring of free drug may be useful for these drugs. Drug shows zero-order elimination kinetics. References for reference ranges: felbamate [32, 33], gabapentin [34], lacosamide [35], lamotrigine [36], levetiracetam [37], oxcarbazepine (10-hydroxyoxcarbazepine metabolite) [38], pregabalin [14], stiripentol [39], tiagabine [40], topiramate [41] and zonisamide [42].

GABAPENTIN:

<table>
<thead>
<tr>
<th>Structure</th>
<th><img src="image" alt="structure" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC Nomenclature</td>
<td>1-(amino methyl)-cyclohexane acetic acid</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C₉H₁₇NO₂</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>171.24 g/mol</td>
</tr>
</tbody>
</table>

Gabapentin was originally approved in 1994 in the United States for the treatment in epilepsy but has achieved greater popularity as an adjunctive therapy for chronic pain. Although structurally related to the neurotransmitter γ-aminobutyric acid (GABA), gabapentin does not appear to interact with GABA receptors in the brain or spinal cord [43]. Gabapentin is rapidly absorbed by the L-amino acid transport system [44]. A study published in 1998 showed a decrease in bioavailability at doses of 4800 mg/day of gabapentin as compared to lower doses, suggesting possible saturability of the L-amino acid transport system [45]. However, a later study demonstrated linear absorption up to 4800 mg/day [46]. Salivary concentrations of gabapentin are only 5–10% those in plasma,
limiting the utility of salivary gabapentin concentrations for TDM \[46\]. Gabapentin is not metabolized and shows little binding to serum proteins \[44\]. The majority of drug is excreted renally and the half-life of the drug increases in renal failure \[43\]. Gabapentin is effectively cleared by hemodialysis \[47\]. A wide range of serum/plasma concentrations are associated with clinical effect \[48\] although effective control of seizures typically requires concentrations above 2 mg/L \[49\]. An approximate reference range of 2–20 mg/L has been proposed \[34\]. Multiple analytical methodologies have been reported for the measurement of gabapentin in plasma/serum including HPLC \[50, 51\], HPLC-tandem mass spectrometry (LC/MS/MS) \[52\], gas chromatography/mass spectrometry (GC/MS) \[53\] and GC/MS/MS \[54\].

**Adverse Effects:**

Gabapentin’s most common side effects in adult patients mainly at higher doses include dizziness, fatigue, weight gain, drowsiness and peripheral swelling of extremities \[55\]. But in the case of children of age group 3-12 years susceptible to mild-to-moderate mood swings, hostility, concentration problems and hyperactivity were observed; also there are rare cases of hepatotoxicity reported in literature \[56, 57\]. Gabapentin should be used carefully in patients with renal impairment due to possible accumulation and toxicity \[58, 59\]. Also overdose have manifested drowsiness, blurred vision, slurred speech and somnolence or coma. Serum Gabapentin concentrations may be measured to confirm diagnosis \[60\].

**Suicide:**

Gabapentin has been associated with an increased risk of suicidal acts or violent deaths \[61\]. In 2009 the USFDA issued a warning of an increased risk of depression, suicidal thoughts and behaviours in patients taking Gabapentin, along with other anticonvulsant drugs \[62\] modifying the packaging insert to reflect this \[55\].

**Withdrawal effects:**

Gabapentin should not be discontinued abruptly after long term use. Abrupt or over rapid withdrawal may provoke a withdrawal syndrome reminiscent to alcohol or benzodiazepine withdrawal \[63, 64\]. Side effects upon discontinuation of Gabapentin that have been reported in medical literature include insomnia, restlessness, agitation,
disorientation, confusion, light sensitivity, diaphoresis, headaches, palpitations, hypertension, chest pain and flu-like symptoms \(^{[63, 65, 66, 67]}\). In one of the case high dose of Gabapentin triggered a seizure in an individual with no history of epilepsy \(^{[66]}\).

Some of the brand names and manufacturer of Gabapentin:

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurostil</td>
<td>Teva Pharma</td>
</tr>
<tr>
<td>Neurontin</td>
<td>Pfizer</td>
</tr>
<tr>
<td>Fanatrex</td>
<td>Fusion Pharmaceuticals</td>
</tr>
<tr>
<td>Gralise</td>
<td>GSK</td>
</tr>
</tbody>
</table>

It is a potent antiepileptic drug. It is used for the treatment of complex partial seizures, with or without secondary generalization in patient over 12 years of age \(^{[68]}\). Gabapentin action is attributed to the irreversible inhibition of the GABA-transaminase enzyme and preventing the degradation of GABA in the brain \(^{[69]}\). Gabapentin is rapidly absorbed after oral administration, and reaches maximal plasma concentrations after 2–3 h. Gabapentin is not metabolized and entirely excreted unchanged in the urine. The drug does not bind to plasma proteins \(^{[69]}\).

Our Literature survey revealed many methods which included kinetic estimation \(^{[70]}\), UV-Vis \(^{[71, 72]}\), fluorescence detector \(^{[73]}\), HPLC methods \(^{[74-76]}\), LC-MS-MS \(^{[77]}\), GC-MS \(^{[77]}\), capillary electrophoresis \(^{[77]}\) for the Gabapentin, for clarity only some of the methods have been discussed here,

H.E. Adelatetf et.al.,\(^{[78]}\) have presented three colorimetric method for determination of Gabapentin in capsules: Method 1: Is based on the reaction of Gabapentin with vanillin (Duquenois reagent) in the presence of McIlvain buffer pH 7.5 and the colour developed was measured at 376 nm. Method 2: Reaction of the primary amino group of Gabapentin with ninhydrin reagent in DMF medium producing a coloured product which absorbs maximally at 569 nm. Method 3: Is based on the reaction of Gabapentin with p-benzoquinone to form a coloured product with maximum absorbance at 369 nm.
A HPLC method for the determination of Gabapentin in human plasma for its application in pharmacokinetic study was developed by Hassan Jalalizadeh et.al. [79]. Gabapentin was quantified using pre-column derivatization with 1-fluoro-2,4-dinitro benzene followed by protein precipitation of plasma with acetonitrile. Amlodipine was used as an internal standard. The column used was Nova-Pak C18, mobile phase being a mixture of 50 mM Sodium dihydrogen orthophosphate (pH-2.5): Acetonitrile (30:70, v/v) with a flow of 1.5 mL/min and detected using UV at 360 nm.

Four methods was published by Sara M. Anis et.al., two methods are based on the reaction of cupric chloride with Gabapentin to form stable complex, which could be measured spectrophotometrically at 246 nm or by using conductromic technique. The other two methods depends on the formation of ion pair complex between Gabapentin and bromothymol blue and bromocresol green respectively which was extracted using methylene chloride [80].

B. Udaykumar Rao et.al developed an isocratic HPLC method for the assay of Gabapentin as bulk and pharmaceutical dosage forms [81]. The mobile phase composition was ammonium dihydrogen orthophosphate buffer and methanol in 60:40 (v/v) proportions. Column used was strong cation exchange column bonded with phenyl sulphonic acid. Quantitation was achieved by UV detection at 200 nm.

A HPLC method using UV detection for the determination of Gabapentin in human plasma was developed by Z.Zhu and L.Neirinck. Gabapentin was extracted from human plasma using reversed-phase solid-phase extraction cartridge followed by derivatization with phenylisothiocynate [77].

In line with USP category I requirement for Gabapentin Abhay Gupta et.al developed an isocratic reversed phase HPLC method for the analysis of dissolution samples of Gabapentin tablets and capsules. The method consisted of Phenomenex Luna Cyano column, methanol:acetonitrile:20 mM sodium dihydrogen orthophosphate (pH of 2.2) in the ratio (5:5:90 v/v) as mobile phase with a flow rate of 1.25 mL/min; both Gabapentin and its major degradation impurity 3,3-pentamethylene-4-butyrolactam was detected using UV at 210 nm [82].
Another Isocratic HPLC method with Brownlee Spheri-5 cyano column using an acetonitrile:10 mM KH$_2$PO$_4$/10 mM KH$_2$PO$_4$ (pH-6.2) in the ratio (8:92 v/v) as mobile phase with a flow rate of 1.0 mL/min. Both the requirements of USP Category I for Gabapentin and USP Category II for major degradation impurity 3,3-pentamethylene-4-butyrolactam was detected using UV at 210 nm $^{[76]}$. 

Gabapentin has no significant ultraviolet, visible or fluorescence absorption. Several HPLC methods for determination of Gabapentin have been published using different derivatizing agent/reagents. Most of the HPLC assay procedures for the determination of Gabapentin are based on the same approach, derivatization followed by HPLC separation in acidic mobile phases and fluorimetric detection. These methods could be optimized for the determination of Gabapentin and its impurities but they suffer some limitations such as a lengthy run time or using special reaction conditions, requires more reagents and it detected at higher UV wavelength, reagent degrades in contact with water, reaction time, higher column temperature, total analysis time which may not be suitable for routine analysis in pharmaceutical industry.

The purity evaluation of Gabapentin in drug substance by determination of related substances is a critical step in examination of the safety and quality of the drug product. Moreover, ICH Guidance Q3A (R2) $^{[83]}$ emphasises the importance of controlling impurities in drug substances.

The comprehensive literatures survey as above revealed that the HPLC methods used for determination of Gabapentin drug substance have low sensitivity, high LOQ and long run time. Moreover, these reported methods have lack of suitable procedure for quantification and estimation of impurities. According to our literature survey, no UHPLC method has yet been reported for the determination and quantification of Gabapentin and its impurities. For the better sensitivity of impurity levels, we have chosen an UHPLC instead of HPLC as this technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity. To the best of our knowledge, determination of these impurities by UHPLC
has not been reported in literature till date. This chapter describes the development, optimization and validation of UHPLC methods for these impurities. Thus, the aim of the present work was to develop and validate a stability indicative reversed phase UHPLC method which is sensitive.

Gabapentin, as officially reported in United state Pharmacopeia (USP) has four potential impurities which needs be identified and quantified and they were identified as Impurity-A, Impurity-B, Impurity-E and Impurity-D (Refer Table 2).

Table 2: Structures of Gabapentin and its impurities with their IUPAC nomenclature, molecular formula and molecular weight.

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
</table>
| Gabapentin           | ![Image](image1.png) | IUPAC Name: 1-(amino methyl)-cyclohexane acetic acid  
Molecular Formula: C₉H₁₇NO₂  
Molecular Weight: 171.24 g/mol |
| Impurity-A           | ![Image](image2.png) | IUPAC Name: [2-aza-spiro[4.5]decan-3-one]  
Molecular Formula: C₉H₁₅NO  
Molecular Weight: 153.22 g/mol |
| Impurity-B           | ![Image](image3.png) | IUPAC Name: [(1-cyano-cyclohexyl)-acetic acid]  
Molecular Formula: C₉H₁₄O₂  
Molecular Weight: 167.21 g/mol |
| Impurity E           | ![Image](image4.png) | IUPAC Name: [carboxymethyl-cyclohexanecarboxylic acid]  
Molecular Formula: C₉H₁₄O₄  
Molecular Weight: 186.21 g/mol |
The content of all these, which could be present at trace levels, is needed to be analyzed in the drug substance. As officially reported in US Pharmacopeia two HPLC method of analysis should be used to identify the impurities such that one method for early eluting impurities namely Impurity-A, Impurity-B, Impurity-E (figure-1) and another method for late eluting impurity namely Impurity-D [84] (figure-2) This necessitated development of a UHPLC method suitable for the separation and simultaneous analysis of Gabapentin and impurities A, B, E and D as a single method of analysis. The method was validated according to ICH Q2 (R1) guideline [85] and also extended to separation of degradation products formed under various stress conditions such as acid hydrolysis, base hydrolysis and oxidative degradation. Photostability is performed according to ICH Q1B guideline [86].
Figure-1: Chromatograms of USP method for Early eluting impurities namely Impurity-A, Impurity-B, Impurity-E
Figure-2: Chromatograms of USP method for late eluting impurities namely Impurity-D.

Experimental:

Chemicals, reagents and standards:

Standard of Gabapentin drug substance was from Biocon Ltd (India) and Gabapentin impurities were procured from USP. Monobasic ammonium phosphate AR grade from Rankem, sodium perchlorate GR from Otto, perchloric acid AR grade from Merck and phosphoric acid AR grade from Fischer were used. Gradient grade
Acetonitrile and Methanol procured from E.Merck, India. Highly pure milli-Q water was collected from Millipore water purification unit.

Instrumentation:

Ultra High Performance Liquid Chromatograph:

Chromatographic separation were performed using UHPLC system with binary solvent manager which can with stand a pressure up to 1200 bar sample manager and PDA detector with Chemstation data handling system. (Agilent 1290 series, Agilent Technologies Inc, USA)

Solutions:

Specificity mixture/System suitability solution: Dissolved and diluted the required quantity of Gabapentin, impurity A, impurity B, impurity E and impurity D to get the concentration of 3.2 mg/mL, 0.72 mg/mL, 0.40 mg/mL, 0.80 mg/mL and 0.05 mg/mL respectively (refer figure-5).

Whilst preparing impurity D solution the stock was prepared by dissolving and making it up with methanol and for further dilution diluent was used.

Sample Solutions:

350 mg of the sample was dissolved and diluted to 25 mL with diluent.

Method development:

In order to achieve a single method for the quantification of all the impurities of Gabapentin, many trials were conducted like variation in the mobile phase composition, change in brand of the column and variation in column oven temperature. Different gradient programmes were used to get a good resolution in short run time. In one of the variations for the gradient programme Table-3 with the run time of 15 minutes (refer figure 3), all the impurities of Gabapentin were well resolved but it was noticed that the re-stabilisation was not effective before the beginning of next injection.

The best results obtained was with the gradient programme as in Table-4, refer figure-4 for the chromatogram of specificity mix. Excellent baseline stabilisation and chromatographic specificity without interference was achieved. Using the described chromatographic conditions, the impurities of Gabapentin were well resolved.
Table-3: Gradient programme

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>6.0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>7.0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>8.0</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>10.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>14.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>14.1</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>15.0</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure-3: Chromatogram of Gabapentin and its impurities with 15 minutes run time.

Table-4: Gradient programme

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>11.0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>12.0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>13.0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>14.0</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>15.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>20.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>22.0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>23.0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure-4: Chromatogram of Gabapentin and its impurities with 23 minutes run time for better restabilisation of the baseline.

Chromatographic Conditions:

Agilent Poroshell 120 EC C8 packing of particle size 2.7 μm having 2.1 mm internal diameter and 100 mm length column was used which was maintained at 40°C, the mobile phase consisted of mobile phase A Buffer pH 1.8 (0.58 g of monobasic ammonium phosphate and 1.83 g of sodium perchlorate in 1000 mL of water pH adjusted with perchloric acid) and acetonitrile (74:26) and mobile phase B of buffer pH 1.8, acetonitrile and methanol (32:35:33) with a flow rate of 0.2 mL/min in a gradient programme as mentioned in Table-4. The injection volume was 2.0 μL with a run time of 23 minutes. Diluent was prepared by dissolving 2.32 g of ammonium phosphate monobasic in 1000 mL of water, pH adjusted to 2.0 with phosphoric acid.

Results:

Forced Degradation:

Forced degradation studies are usually the most important part of the drug development strategy being undertaken to elucidate the intrinsic stability of the drug substance. Such studies are therefore conducted under more severe and exaggerated conditions than those usually used for long-term stability tests. The information gathered
may help establishing the drug degradation pathway as well as development and validation of the suitable analytical procedures.

Forced degradation studies of drug substance included appropriate solid state and solution state stress conditions in accordance with the ICH regulatory guidance. When Gabapentin sample was subjected to the solid state degradation for about 72 hr at different conditions, there was no noticeable rise in unknown or known impurities except variations found in the content of Impurity A. Refer Figure-5 for the variations in the content of impurity A at different solid stress condition.

![Figure 5: Trend chart for the variations in the content of impurity A at different solid stress conditions.](image)

![Figure 6: Trend chart of the percent content of Gabapentin drug substance at different liquid stress conditions.](image)

When Gabapentin sample was subjected to the liquid state degradation at different conditions, degradation was not observed with acidic medium. Further in basic medium the degradation was enormous which was uncontrollable and when sample was subjected to oxidative degradation massive degradation resulting in many impurities. Refer figure-6 for the trend chart of the percent content of Gabapentin drug substance in liquid state stress condition. Refer figure-7 for the overlay of peroxide blank and peroxide treated sample chromatograms.
Method Validation:

The developed method was validated as per the ICH guidelines, the following parameters were covered specificity, precision, LOD and LOQ, linearity, range, accuracy, robustness and solution stability.

Specificity:

Specificity is the ability of a method to unequivocally assess the analyte in the presence of component that may be expected to be present as a residue such as impurities and degradation products.

All the impurities of Gabapentin were injected individually to confirm the retention time. Diluent was injected as blank to prove that there was no interference from the diluent at the retention time corresponding to Gabapentin and its impurity. The relative retention time (RRT) of the impurity E, impurity A, impurity B and impurity D with respect to Gabapentin is 2.00, 2.32, 2.58 and 10.53 respectively.

Precision:

Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision is usually expressed as standard deviation or
relative standard deviation of a series of measurements. The precision demonstrates the degree of reproducibility or repeatability of an analytical method under normal operating conditions.

**System Precision:**

The percent related standard deviation for replicate injections of Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D were 0.08, 0.68, 0.42, 0.81 and 0.12 respectively. The results prove the precision of the system effectively.

**Method Precision:**

Replicate preparations of the sample was prepared as per the methodology and injected. The percent relative standard deviation for the percent contents of Impurity A is 0.68. The results prove the precision of the method effectively.

**Limit of Detection (LOD) and Limit of Quantification (LOQ):**

LOQ is the lowest amount of analyte in the sample that can be determined with acceptable precision and accuracy. LOD is the lowest amount of analyte in the sample that can be detected, but not necessarily quantitated under the stated experimental conditions.

The method of signal to noise ratio (S/N) was used to calculate LOD and LOQ. In the case of LOQ for Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D the concentration at which the S/N value of around 10 was achieved were 0.014 mg/mL, 0.00098 mg/mL, 0.006 mg/mL, 0.004 mg/mL and 0.00014 mg/mL respectively. The %RSD for the precision at these concentrations was 0.98, 1.2, 0.8, 1.4 and 1.8 for Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D respectively.

LOD for Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D was achieved at 0.0046 mg/mL, 0.0003 mg/mL, 0.002 mg/mL, 0.0012 mg/mL and 0.000046 mg/mL concentration respectively, where S/N was around 3.

**Linearity and Range:**

The linearity of an analytical method is the ability of the analytical method to elicit test results that are proportional to the concentration of analyte in the sample within the range.

Linearity for Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D were established for 5 concentration levels including LOQ. The data was evaluated using
statistical analysis like slope, intercept and correlation coefficient figure-8. Six replicate injection of selected four levels were injected to prove the precision of the range, and %RSD for the same was calculated (Table-5). The linearity graph was plotted area (y-axis) versus concentration (x-axis).

**Figure-8: Linearity plot for Gabapentin and its impurities.**
Table-5: Range with % RSD for Gabapentin and its impurities.

<table>
<thead>
<tr>
<th>Gabapentin</th>
<th>Levels</th>
<th>LOQ</th>
<th>Level-1</th>
<th>Level-2</th>
<th>Level-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration mg/mL</td>
<td>0.014</td>
<td>1.4</td>
<td>7</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>0.98</td>
<td>0.81</td>
<td>0.12</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

| Impurity A | Concentration mg/mL | 0.00098 | 0.0098 | 0.015 | 0.03 |
| %RSD | 1.2 | 1.1 | 0.9 | 0.7 |

| Impurity B | Concentration mg/mL | 0.006 | 0.009 | 0.012 | 0.024 |
| %RSD | 0.8 | 0.9 | 0.7 | 0.2 |

| Impurity E | Concentration mg/mL | 0.004 | 0.008 | 0.02 | 0.2 |
| %RSD | 1.4 | 1.2 | 0.9 | 0.6 |

| Impurity D | Concentration mg/mL | 0.00014 | 0.0014 | 0.0028 | 0.006 |
| %RSD | 1.8 | 1.3 | 0.8 | 0.6 |

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This parameter demonstrates the capability of the method to give accurate results.

Accuracy for Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D was performed in triplicate for 4 levels including LOQ. The percentage recovery was found to be between 93 and 103. The accuracy results were summarized in Table-6.
Table 6: Results of accuracy study.

<table>
<thead>
<tr>
<th></th>
<th>Spiked</th>
<th>Replicate 1</th>
<th>Recovery (%)</th>
<th>Replicate 2</th>
<th>Recovery (%)</th>
<th>Replicate 3</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>0.00098</td>
<td>0.00095</td>
<td>96.94</td>
<td>0.00097</td>
<td>98.98</td>
<td>0.00095</td>
<td>96.94</td>
</tr>
<tr>
<td>L-1</td>
<td>0.0098</td>
<td>0.0096</td>
<td>97.96</td>
<td>0.0095</td>
<td>96.94</td>
<td>0.0094</td>
<td>95.92</td>
</tr>
<tr>
<td>L-2</td>
<td>0.015</td>
<td>0.0140</td>
<td>93.33</td>
<td>0.0144</td>
<td>96.00</td>
<td>0.0146</td>
<td>97.33</td>
</tr>
<tr>
<td>L-4</td>
<td>0.030</td>
<td>0.031</td>
<td>103.33</td>
<td>0.029</td>
<td>96.67</td>
<td>0.029</td>
<td>96.67</td>
</tr>
<tr>
<td>Impurity B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>0.006</td>
<td>0.0057</td>
<td>95.00</td>
<td>0.0056</td>
<td>93.33</td>
<td>0.0057</td>
<td>95.00</td>
</tr>
<tr>
<td>L-1</td>
<td>0.009</td>
<td>0.0087</td>
<td>96.67</td>
<td>0.0088</td>
<td>97.78</td>
<td>0.0085</td>
<td>94.44</td>
</tr>
<tr>
<td>L-2</td>
<td>0.012</td>
<td>0.012</td>
<td>100.00</td>
<td>0.0116</td>
<td>96.67</td>
<td>0.0118</td>
<td>98.33</td>
</tr>
<tr>
<td>L-4</td>
<td>0.024</td>
<td>0.0245</td>
<td>102.08</td>
<td>0.0235</td>
<td>97.92</td>
<td>0.0248</td>
<td>103.33</td>
</tr>
<tr>
<td>Impurity E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>0.004</td>
<td>0.0038</td>
<td>95.00</td>
<td>0.0039</td>
<td>97.50</td>
<td>0.0041</td>
<td>102.5</td>
</tr>
<tr>
<td>L-1</td>
<td>0.008</td>
<td>0.0077</td>
<td>96.25</td>
<td>0.0078</td>
<td>97.50</td>
<td>0.0079</td>
<td>98.75</td>
</tr>
<tr>
<td>L-2</td>
<td>0.02</td>
<td>0.0192</td>
<td>96.00</td>
<td>0.0194</td>
<td>97.00</td>
<td>0.0188</td>
<td>94.00</td>
</tr>
<tr>
<td>L-4</td>
<td>0.2</td>
<td>0.205</td>
<td>102.50</td>
<td>0.197</td>
<td>98.50</td>
<td>0.200</td>
<td>100.00</td>
</tr>
<tr>
<td>Impurity D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>0.00014</td>
<td>0.000138</td>
<td>98.57</td>
<td>0.000136</td>
<td>97.14</td>
<td>0.000137</td>
<td>97.86</td>
</tr>
<tr>
<td>L-1</td>
<td>0.0014</td>
<td>0.00136</td>
<td>97.14</td>
<td>0.00138</td>
<td>98.57</td>
<td>0.00135</td>
<td>96.43</td>
</tr>
<tr>
<td>L-2</td>
<td>0.0028</td>
<td>0.00278</td>
<td>99.29</td>
<td>0.00276</td>
<td>98.57</td>
<td>0.00281</td>
<td>100.36</td>
</tr>
<tr>
<td>L-4</td>
<td>0.006</td>
<td>0.0058</td>
<td>96.67</td>
<td>0.0057</td>
<td>95.00</td>
<td>0.0056</td>
<td>93.33</td>
</tr>
</tbody>
</table>

Robustness:

The robustness of an analytical method is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during routine usage. The robustness of the method was demonstrated by altering experimental conditions and chromatographic resolution between impurity A and impurity B (figure-9). The deliberate changes were made in the chromatographic conditions, viz. change in flow rate by ±0.05 mL/min and change in the column temperature ± 5°C.
Solution Stability:

The solution stability is demonstrated to check the ability of the solution to remain stable after its preparation by not affecting the analytical results obtained for the solution in relation to its initial data and/or by not forming degradation products which would cause a variation in the evaluation of data.

Solution stability is performed by carrying out analysis for a set of solution preparations of sample by injecting at T₀ (Initial) and at frequent intervals maintained at room temperature, after its preparation. It is the evaluation of time interval within which the sample solution remains stable without undergoing any significant changes. The sample solution was found to be stable up to 24 hr without any increase in the impurity level and formation of new impurities. But after 24 hr impurity A was increased.

Discussion:

Primarily the objective was to develop a single method for the quantification of known impurities of Gabapentin which is stability indicating, cost effective and rugged and which can be used for the routine analysis. Several trials was performed in order to reduce the run time, in one of the variations for the gradient programme with the run time of 15 minutes, all the impurities of Gabapentin were well resolved but it was noticed that the baseline was not re-stabilising in the beginning of the next run.
In order to streamline the baseline stabilization the gradient programme was extended to 23 minutes, without change in the resolution pattern and this method was validated. Gabapentin and its impurities were well resolved with no interference from the blank and mobile phase. In the precision study the % RSD for replicates was found less than 2.0 for all the impurities of Gabapentin.

The described method was linear for the range (Table-6) of each impurity. The value of correlation coefficient for each impurity was greater than 0.999. The LOQ concentration for Gabapentin, impurity A, impurity B, impurity E and impurity D were 0.014 mg/mL, 0.00098 mg/mL, 0.006 mg/mL, 0.004 mg/mL and 0.00014 mg/mL respectively. The method showed excellent recovery at four different studied concentrations for all the impurities, the overall recoveries were between 93 to 103%.

The chromatographic resolution between impurity A and impurity B was used to evaluate the method robustness under modified conditions. There was no significant change in the resolution under all specified conditions tested, demonstrating sufficient robustness.

The increase in the content of impurity A after 24 hr led to the conclusion that the sample solution is stable up to 24 hr.

In order to assure the selectivity and provide an indication of the stability-indicating properties of the proposed method, forced degradation studies were performed under two categories. In the case of solid state degradation the sample was exposed to 25°C/60%RH, 25°C/90% RH, 30°C/65% RH, 40°C/75% RH, 80°C and UV. In these cases it was found that only impurity A content was raising compared to the initial value. In the case of liquid state Gabapentin sample was subjected to acidic, basic and oxidative degradation.

Degradation was not observed when sample was subjected to acidic hydrolysis, but when the sample was subjected to basic hydrolysis degradation was uncontrollable to the extent of decrease in the Gabapentin content to only 4%. Under oxidative stress condition the degradation was high resulting in more number of degradation products.
A reversed phase simple and sensitive UHPLC method was developed and validated for the identification and quantification of Gabapentin and its impurities. The development of method was done with due considerations on the runtime, resolution of the impurities, reproducibility and cost of analysis. The USP official monograph for estimation of impurities in Gabapentin consists of two methods one for the early eluting impurities and the other for late eluting impurities. The method developed will accommodate both early and late eluting impurities in a single run with good resolution and sensitivity. This was achieved with Agilent Poroshell 120 EC C8 2.7 µm 2.1 x 100 mm column maintained at 40°C with mobile phase consisting of buffer having a pH of 1.8, acetonitrile and methanol in gradient mode at a flow rate of 0.2 mL/min, detected at 215nm and with the injection volume of 2.0 µL. The chromatographic resolution between impurity A and impurity B were found to be greater than 2.3. The responses were determined and regression coefficient values were obtained greater than 0.999 for Gabapentin and its impurities. The concentration of Gabapentin, Impurity A, Impurity B, Impurity E and Impurity D were 0.014 mg/mL, 0.00098 mg/mL, 0.006 mg/mL, 0.004 mg/mL and 0.00014 mg/mL respectively at which LOQ was achieved. The drug substance was subjected to stress conditions as prescribed by the ICH. Degradation was found to occur under oxidative stress condition. The test solution was found to be stable up to 24 hr. It was proved that the method is stability indicative with the support of forced degradation experiments.

A single, simple, sensitive, cost effective and stability indicating UHPLC method has been developed for quantitative determination of impurities in Gabapentin drug substance. The proposed method has been successfully validated for specificity, precision, linearity, range, accuracy, robustness and solution stability. One of the major advantages of the method is that all the known impurities of Gabapentin drug product can be quantified in one method, compared to two methods as per USP monograph of Gabapentin. Instead of conventional HPLC methods one can use the proposed UHPLC method in the routine and stability analysis of Gabapentin drug substance.
References:


36. Bartoli, A. Guerrini, R. Belmonte, A.; Alessandri, M.G. Gatti, G. Perucca, E. The influence of dosage, age, and comedication on steady state plasma lamotrigine


60. R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, Foster City, CA, 2008, 8, 677–678.


85. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures; Text and Methodology Q2 (R1), November 2005, Step 4 version.