PREFACE
Chemical analysis has become indispensable in many areas other than traditional chemistry. Chemical analysis is now used in the medical sciences (for diagnostics), in biochemistry, food chemistry, environmental sciences, in numerous industrial areas and in pharmaceutical industry, where about 95% of the drugs are of synthetic origin. Active pharmaceutical substance means drug in formulation i.e. the drug is not administered in its pure form, as was done earlier, but is administered as tablets, capsules, injections and syrups with several excipients. This means that methods of assay should be compatible with the presence of excipients.

The need for expeditious and reliable testing is increasing in the field of medicinal formulations. Presently in the pharmaceutical industry; drug analysis plays a vital role in deciding the quality and potency of the drug substances. The selection of analytical method used to determine the active ingredients of the drugs and impurities in the formulation is a challenging problem. The method should be sensitive, accurate, rapid, precise, reproducible and free from the interferences of the excipients used in the formulation.

Stability indicating analytical methods are developed to monitor the stability of active pharmaceutical ingredients and pharmaceutical dosage forms during the investigational phase of drug development, and once the drug is marketed for the ongoing stability studies which must be conducted. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of atmospheric factors such as temperature, humidity and light, enables recommended storage conditions, re-test periods and shelf lives to be established. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help, to establish the degradation pathways and the intrinsic stability of the molecules and to validate the stability indicating power of the analytical procedures used.

For the drug to be manufactured in pure form, it is important to keep a strict vigil on quality at all stages of its manufacture and market distribution. The impurities are confined to those arising from incomplete and side reactions, ingredient due to chemical conditions (i.e. oxidation, reduction, hydrolysis etc). This is necessary to develop organo-analytical chemistry, not only for the assay of a drug/ chemical but also for determining its impurity profile. In pharmaceutical industry, prime
importance is given to separation of ingredient from the above medical formulations, which can be defined as the sum of all the factors, which contribute directly or indirectly to the safety, efficacy, reliability and reproducibility of the product.

By keeping the importance of the active pharmaceutical ingredient organic impurities quantification method, the author has proposed to quantify organic impurities in some of important anti retroviral and antihypertensive pharmaceutical substances, which are used for treating AIDS, seizures, bipolar disorder and hypertension. The author selected the following potential active pharmaceutical drugs substances. The literature survey reveals that there is no rapid resolution stability indicative quantification methods for organic impurities for the active pharmaceutical ingredients mentioned in preface Table T1 with the details of their chemical names, structure and therapeutic action.

**Preface. Table T1:**

<table>
<thead>
<tr>
<th>API</th>
<th>Therapeutic</th>
<th>IUPAC NAME</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stavudine</td>
<td>Antiretroviral; for HIV Aids</td>
<td>1-[(2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methyl-1,2,3,4-tetrahydroprimidine-2,4-dione</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>Nevirapine</td>
<td>Antiretroviral; Reverse Transcriptase Inhibitor.</td>
<td>11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Anticonvulsant</td>
<td>1,2-benzisoxazole-3-methanesulfonamide</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
### API | Therapeutic action | IUPAC NAME Structure
--- | --- | ---
Quetiapine | Antipsychotic used in treatment of bipolar disorder | 2-((4-dibenzo[h][1,4]thiazepine-11-yl-1-piperazinyl)ethoxy)ethanol
Ramipril | Anti-hypertensive. | (2S,3αS,6αS)-1-((2S)-2-{{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl}octahydrocyclopenta[h]pyrrole-2-carboxylic acid

**AIDS (Acquired immune deficiency syndrome or acquired immunodeficiency syndrome)** is a disease of the human immune system caused by the human immunodeficiency virus (HIV). AIDS is now a pandemic. In 2007, it was estimated that 33.2 million people lived with the disease worldwide, and that AIDS had killed an estimated 2.1 million people, including 330,000 children. Over three-quarters of these deaths occurred in sub-Saharan Africa, retarding economic growth and destroying human capital.

This condition progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors. HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, pre seminal fluid, and breast milk.

This transmission can involve anal, vaginal or oral sex, blood transfusion, contaminated hypodermic needles, exchange between mother and baby during pregnancy, childbirth, or breastfeeding, or other exposure to one of the above bodily fluids.
Seizures can cause involuntary changes in body movement or function, sensation, awareness, or behavior. A seizure can last from a few seconds to status epileptics, a continuous seizure that will not stop without intervention. Seizures are often associated with a sudden and involuntary contraction of a group of muscles and loss of consciousness. However, a seizure can also be as subtle as marching numbness of a part of the body, a brief or long term loss of memory, sparkling or flashes, sensing/discharging of an unpleasant odor similar to alcohol base being produced by internal organs, a strange epigastric sensation or a sensation of fear and total state of confusion which in some cases leads to death during seizure. After a heavy seizure attack, since the brain is recovering, there is a sudden loss of memory; usually the short term memory.

Bipolar disorder disorder has been subdivided into bipolar I, bipolar II, cyclothymia, and other types, based on the nature and severity of mood episodes experienced; the range is often described as the bipolar spectrum.

Bipolar disorder also called as manic depression is a psychiatric diagnosis that describes a category of mood disorders defined by the presence of one or more episodes of abnormally elevated mood clinically referred to as mania or, if milder, hypomania. Individuals who experience manic episodes also commonly experience depressive episodes or symptoms, or mixed episodes in which features of both mania and depression are present at the same time.

These episodes are usually separated by periods of "normal" mood, but in some individuals, depression and mania may rapidly alternate, known as rapid cycling. Extreme manic episodes can sometimes lead to psychotic symptoms such as delusions and hallucinations.

The onset of full symptoms generally occurs in late adolescence or young adulthood. Diagnosis is based on the person's self-reported experiences, as well as observed behavior. Episodes of abnormality are associated with distress and disruption, and an elevated risk of suicide, especially during depressive episodes. In some cases it can be a devastating long-lasting disorder; in others it has also been associated with creativity, goal striving and positive achievements.
Data from the United States on lifetime prevalence vary but indicate a rate of around 1 percent for Bipolar I, 0.5 to 1 percent for Bipolar II or cyclothymia, and between 2 and 5 percent for sub threshold cases meeting some but not all criteria.

**Hypertension** also referred to as high blood pressure, HTN or HPN, is a medical condition in which the blood pressure is chronically elevated. In current usage, the word "hypertension" without a qualifier normally refers to systemic, arterial hypertension.

Hypertension can be classified as either essential (primary) or secondary. Essential hypertension indicates that no specific medical cause can be found to explain a patient's condition. About 95% of hypertension is essential hypertension. Secondary hypertension indicates that the high blood pressure is a result of (i.e., secondary to) another condition, such as kidney disease or tumors (adrenal adenoma or pheochromocytoma).

Hypertension is one of the most common complex disorders. The etiology of hypertension differs widely amongst individuals within a large population.

Hypertension may be secondary to other diseases but over 90% of patients have essential hypertension which is of unknown origin. It is observed though that:

- Having a personal family history of hypertension increases the likelihood that an individual develops
- Essential hypertension is four times more common in black than white people, accelerates more rapidly and is often more severe with higher mortality in black patients.

In view of the above facts the author has carried out the present study on the three therapeutic categories i.e, antiretroviral, anticonvulsant and on anti hypertensive. The above active pharmaceutical ingredients are invariable for studies of organic impurities quantification by sensitive, fast and reproducible monitoring methods due to the following points
• Pure active pharmaceutical substance is to be consumed by patient for its intended therapeutic usage.

• Organic impurities present in active pharmaceutical substance can cause unpredicted side effects to patients, which some times may be fatal.

• High percentages of impurities will reduce the active pharmaceutical substance content in tablets by % there by damaging the action in terms of time or healing.

The literature reveals that there is no attempt done to quantify organic impurities through stability indicative methods by rapid resolution liquid chromatography of the above cited active pharmaceutical ingredients. Hence the author in the present investigation have been attempted quantification of impurities through stability indicative methods by sophisticated, fast method by Rapid Resolution Liquid Chromatography (RRLC) for active pharmaceutical ingredients stated above.

Literature survey till date revealed that there are no rapid resolution liquid chromatographic methods which are stability indicative available for the above stated drugs. All the existing methods are developed by isocratic and gradient separation and with in time of 30 to 75 minutes for organic impurities separation and assay estimation. The proposed rapid resolution liquid chromatographic (RRLC) methods are simple isocratic methods with in very short time (5-15 minutes). These methods will save lot of valuable time and costly chemicals and will help in fast quick analysis and fast release of batches. All the methods are tested for their stability indicative nature by stress studies and duly validated.

In this context the author in present research work investigated with a prime selective method of rapid resolution liquid chromatography technique for quantification of impurities in active pharmaceutical ingredients. Many analyses now carried out daily in the clinical, agricultural, biological, food and environmental sciences, and forensic laboratories all over the world involve separation as an integral part of the procedure. Due to recognition of the importance of these techniques to the development of the chemical and biochemical sciences has increasingly dawned on...
scientists in these fields only in recent times. For the above facts the author in the present investigation has been selected RRLC as a tool for research program.

In the view of importance of “Stability indicative methods for identification and quantification of organic impurities” and “Stability indicative methods for assay estimation” of antiretroviral, anticonvulsant, and on anti hypertensive active pharmaceutical ingredients of the author has carried out in the present investigation and the thesis has been designed in the following manner.

This thesis is divided into nine Chapters:

**Chapter 1** Discussion on the importance of stability indicating analytical methods for analyzing of active pharmaceutical ingredients and organic impurities in it.

**Chapter 2** Deals with the fundamental steps of RRLC and gives detailed information of Active pharmaceutical ingredients and theoretical information of HIV Aids, siezure, bipolar disorder and hypertension.

**Chapter 3** Demonstration of performance and efficacy of RRLC technique over the conventional HPLC technique.

**Chapter 4** Explains “Stability indicating Fast LC method for analyzing the impurities and assay of Stavudine”.

**Chapter 5** Focuses on “Stability indicating Fast LC method for analyzing the impurities and assay of Nevirpaine”.

**Chapter 6** Deals with “Stability indicating Fast LC method for analyzing the impurities and Assay of Zonisamide”.

**Chapter 7** Discussion on “Stability indicating fast LC method for analyzing the impurities and assay of Quetiapine”.

**Chapter 8** Deals with “Stability indicating Fast LC method for analyzing the impurities and Assay of Ramipril”

**Chapter 9** Summary of all the methods and conclusion of the study.
Table (Preface Table T2) gives in brief the list of drugs dealt with in the present work together with their chemical families and therapeutic actions.

### Table T2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical family</th>
<th>Therapeutic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stavudine</td>
<td>Dipyridodia-zepinone</td>
<td>Antiretroviral for HIV Aids</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>Dipyridodia-zepinone</td>
<td>Antiretroviral; Reverse Transcriptase Inhibitor.</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Sulfonamide</td>
<td>Anticonvulsant.</td>
</tr>
<tr>
<td></td>
<td>antiseizure agent</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Combined serotonin (5HT&lt;sub&gt;2&lt;/sub&gt;) and dopamine (D&lt;sub&gt;2&lt;/sub&gt;) receptor antagonist.</td>
<td>Antipsychotic uses in treatment of Bipolar disorder</td>
</tr>
<tr>
<td>Ramipril</td>
<td>Angiotensin converting enzyme (ACE) inhibitor; converted to active, diacid metabolite, ramiprilat.</td>
<td>Antihypertensive</td>
</tr>
</tbody>
</table>

Literature survey for the selected drugs revealed that there are no fast RRLC method for determination of impurities and assay for the following active pharmaceutical ingredients, i.e. Stavudine, Nevirapine, Zonisamide, Quetiapine and Ramipril.

Therefore the above active pharmaceutical ingredients have been taken for the present research work aims at development of novel methods for the isocratic separation and simultaneous assay of the drugs mentioned.