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# VARIOUS QUANTITATIVE & QUALITATIVE METHODS FOR ESTIMATION OF ANTI-EPILEPTIC DRUGS IN BULK DRUG, FORMULATIONS AND THEIR BIOLOGICAL SAMPLES –A REVIEW

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## ABSTRACT

This paper describes the various analytical methods reported for quantitative estimation of various drugs used in the treatment of Epilepsy. Quantification of drugs in bulk drug, active pharmaceutical ingredients and their formulation and their impurity profiling is mandatory as per the requirement of governing bodies. Estimation of the drug samples in biological samples is of prime requirement for in Vitro and in vivo studies of bio availability and bio equivalence studies. This paper includes all the analytical methods and bio-analytical methods reported for estimation of various combinations of anti-epileptic drugs in biological and non-biological fluids.

**KEYWORDS:** Anti-Epileptic drugs; Therapeutic drug monitoring; Estimation; Quantitative.

## 1. INTRODUCTION

Epilepsy is the condition characterized by recurrent episodes of seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons. Antiepileptic drugs are a diverse group of pharmacological agents used in the treatment of epileptic seizures. Anticonvulsants are also increasingly being used in the treatment of disorder and disorder, since many seem to act as mood stabilizers, and for the treatment of neuropathic pain.

**Classification of Seizures**

**1) Generalized seizures**

a. **Tonic Clonic (Grand mal):** Its characterized by tonic rigidity, followed in 15-30 sec by a relaxation phase. As the relaxation phase becomes longer, the attack enters the clonic phase with massive jerking over 60-120 sec. Effective drugs include Phenytoin, Phenobarbital, Primidone, Carbamazepine.

b. **Absence:** Its characterized by both sudden onset and abrupt cessation, its duration is usually less than 10 secs, it occurs in children. Effective drugs include Ethosuximide, Trimethadione, Clonazepam, Valproic Acid.

c. **Myoclonic:** It consists of myoclonic jerking and it occurs in a variety of seizures. Effective drugs include Phenobarbital, Clonazepam, Valproic Acid

d. **Atonic:** It is characterized by sudden loss of muscle tone lasting 10-60 secs. Effective drugs include Diazepam, Clonazepam.

e. **Infantile spasma:** It is characterized by brief, recurrent myoclonic jerking with Abrupt flexion/extention of limbs or whole body. Effective drugs include Diazepam, Clonazepam, Phenobarbital, Corticotrophin.

**2) Partial seizures**

a. Simple partial seizures: It is characterized by minimal spread of abnormal discharge with consciousness. Clonic jerking last for 30-120secs.Effective drugs include Primidone, Clorazepate, Carbamazepine, Valproic Acid.

b. Complex partial seizures: It is temporal lobe epilepsy and neuronal discharge widespread to limbic system, alteration of consciousness, last for 30-120 secs.Effective drugs include Carbamazepine, Primidone, Phenytoin.

**Table 1: List of Marketed Drugs.**

| S. No. | Drugs | Brand names with strength |
|---|---|---|
| 1 | Acetazolamide | Tab DIAMOX 250; Cap “DIAMOX” or “D3” |
| 2 | Lorazepam | Tab ATIVAN 0.5, Tab ATIVAN 1, Tab ATIVAN 2 |
| 3 | Carbamazepine | Cap CARBATROL 100, Cap Carbatrol 200, Cap Carbatrol 300 Cap Tegretol 200, Tab TEGRETOL 100 |
| 4 | Valproate | Cap DEPAKENE 250 |
| 5 | Phenytoin | Tab DILATIN 50, Cap DILATIN 30, Cap DILATIN 100 |
| 6 | Tiagabine | Tab GABITRIL 2, Tab GABITRIL 4, Tab GABITRIL 12, Tab GABITRIL 16 |
| 7 | Levetiracetam | Tab KEPPRA 250, Tab KEPPRA 500, |

| | | |
|---|---|---|
| | | Tab KEPPRA 750, Tab KEPPRA 1000 |
| 8 | Clonazepam | Tab KLONOPIN 0.5, Tab KLONOPIN 1, Tab KLONOPIN 2 |
| 9 | Lamotrigine | Tab chewable LAMICTAL 25, Tab LAMICTAL 200 |
| 10 | Pregabalin | Cap LYRICA 100, Cap LYRICA 150, Cap LYRICA 200, Cap LYRICA 300 |
| 11 | Primidone | Tab MYSOLINE 225 |
| 12 | Gabapentin | Tab NEURONTIN 600, Tab NEURONTIN 800, Cap NEURONTIN 100, Cap NEURONTIN 300, Cap NEURONTIN 400 |
| 13 | Phenobarbital | Tab PHENOBARBITAL 100 |
| 14 | Vigabatrin | Tab SABRIL 500 |
| 15 | Topiramate | Tab TOPAMAX 200, Cap TOPAMAX 25 |
| 16 | Clorazepate | T-Tab TRANXENE 15, SD TRANXENE 22.5 |
| 17 | Oxcarbazepine | Tab TRILEPTAL 600 |
| 18 | Ethosuximide | Cap ZARONTIN 250 |

## 2. Description

### 2.1. Phenobarbital, Phenytoin, Primidone, carbamazepine, Lamotrigine, Oxcarbazepine and Licarbazepine

Serralheiro, A et al. developed a method for simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma with the title "First HPLC-UV method for rapid and simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma", the details of method is described as follows: A sensitive and fast high-performance liquid chromatographic method coupled with ultraviolet detection is herein reported for the simultaneous determination of human plasma concentration of six antiepileptic drugs frequently used in clinical practice [phenobarbital (PB), primidone (PRM), phenytoin (PHT), carbamazepine (CBZ), lamotrigine (LTG), oxcarbazepine (OXC)] and some of their main metabolites, carbamazepine-10,11-epoxide (CBZ-E), 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine (trans-diol) and licarbazepine (Lic). Sample preparation consisted of a deproteinization step with methanol followed by a solid-phase extraction procedure. Chromatographic separation was achieved in approximately 15 min on a reversed-phase C18 column using a mobile phase composed by water-methanol-acetonitrile-triethylamine (68.7:25:6:0.3, v/v/v/v; pH 6.5) pumped isocratically at 1.0 mL/min. The detector was set at 237 nm. Calibration curves were linear with regression coefficients greater than 0.992 over the concentration ranges 0.25-100 µg/mL for PB, 0.4-50

µg/mL for PRM, 0.5-50 µg/mL for PHT, 0.1-50 µg/mL for CBZ, LTG and CBZ-E, 0.1-25 µg/mL for OXC, 0.25-10 µg/mL for trans-diol and 0.15-80 µg/mL for Lic. Inter- and intra-day imprecision did not exceed 12.15% and inaccuracy was within ±14.91%. Absolute mean recoveries ranged from 78.49 to 101.04% and no interferences were observed at the retention times of the analytes and internal standard (ketoprofen). This bioanalytical method was successfully applied to real plasma samples from epileptic patients and it seems to be a suitable tool for routine therapeutic drug monitoring and also to support other clinical pharmacokinetic-based studies.[1]

**2.2. Phenobarbital and Phenytoin**

Amiri Pebdani, et al. developed a method for liquid-phase microextraction based on sequential injection solidified floating organic drop combined with HPLC for the determination of phenobarbital and phenytoin with the title "Modified dispersive liquid-phase microextraction based on sequential injection solidified floating organic drop combined with HPLC for the determination of phenobarbital and phenytoin", the details of method is described as follows: A modified dispersive liquid phase microextraction based on sequential injection solidified floating organic drop was developed for simultaneous separation/preconcentration of trace amounts of phenobarbital and phenytoin. The important factors affecting on the extraction recovery including pH, the volume of extraction solvent, ionic strength, and the number of injections were investigated and optimized by Box–Behnken design and desirability function. Under the optimum experimental conditions, the calibration graph was linear in the concentration range of 1.0–300.0 µg/L ($r^2 = 0.997$) for phenobarbital and 2.0–400.0 µg/L ($r^2 = 0.996$) for phenytoin. The limit of detection and limit of quantification were 0.35 and 1.2 µg/L for phenobarbital and 0.65 and 2.2 µg/L for phenytoin, respectively. The relative standard deviation for six replicate determinations at 10µg/L was 3.3 and 4.1% for phenobarbital and phenytoin, respectively. The developed method was successfully applied to the determination of phenobarbital and phenytoin in urine and plasma samples.[2]

**2.3. Phenytoin (Method-I)**

S. M. N. Roy, et al. developed a method for Quantification of Phenytoin in Human Plasma by Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry with the title "Determination and Quantification of Phenytoin in Human Plasma by Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry", the details of the

method is described as follows: A rapid and simple high pressure liquid chromatography method with mass spectrometry detection was developed and validated for the determination of phenytoin in human plasma. Metaxalone was used as internal standard. The sample preparation involves a rapid and simple procedure based on liquid-liquid extraction. Analysis was performed in less than 3.0 minutes in isocratic mode on a reversed phase C18 column (5µ; 50 × 4.6 mm) using a mobile phase composed of acetonitrile-buffer 2 mm ammonium acetate (80:20 v/v), pH of buffer adjusted to 3.4 using formic acid, at 0.4 mL min-1 flow rate. The calibration curves were linear in the measured range between 101.2 ng mL-1 and 5060.0 ng mL-1. The validated lowest limit of quantification was 101.2 ng mL-1 for phenytoin. The mean relative recovery for drug and Internal standard was found to be 78.33% and 77.04%, respectively. The described method has the advantage of being rapid and easy and it could be applied in therapeutic monitoring of these drugs in human plasma.[3]

**2.4. Levetiracetam, Phenytoin, and Valproate**

Nissen-Meyer, et al. developed for a method for Levetiracetam, Phenytoin, and Valproate Acting Differently on Rat Bone Mass, Structure, and Metabolism with the title "Levetiracetam, Phenytoin, and Valproate Act Differently on Rat Bone Mass, Structure, and Metabolism" the details of the method is described as follows: Long-term treatment with antiepileptic drugs (AEDs) is associated with increased risk of fractures. Phenytoin (PHT) and valproate (VPA) have both been suggested to influence bone health, whereas levetiracetam (LEV) is scarcely studied. VPA induced increased bone turnover, whereas modest changes were observed for PHT. Interestingly, low-dose LEV was associated with reduced biomechanical strength of the femoral neck (mainly trabecular bone). In addition, low-dose LEV treatment resulted in significantly reduced levels of serum osteocalcin, a marker of bone formation. Histomorphological analyses indicated increased retention of cartilage remnants at the growth plate metaphysis of rats treated with low-dose LEV vs. controls. PHT, VPA, and LEV exert differential effects on bone mass and strength, suggesting different mechanisms of action. The weakening effect of low-dose LEV on the femoral neck, despite a constant BMD, suggests a primary effect on bone quality. These findings warrant further human studies of possible adverse effects of LEV on bone development and growth, particularly in children and adolescents.[4]

**2.5. Carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbital**

Queiroz, R. H., et al. developed a method for Quantification of carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbital in plasma samples by stir bar-sorptive extraction and liquid chromatography with the title "Quantification of carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbital in plasma samples by stir bar-sorptive extraction and liquid chromatography "the details of the method is described as follows: A sensitive and reproducible stir bar-sorptive extraction and high-performance liquid chromatography-UV detection (SBSE/HPLC-UV) method for therapeutic drug monitoring of carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbital in plasma samples is described and compared with a liquid:liquid extraction (LLE/HPLC-UV) method. Important factors in the optimization of SBSE efficiency such as pH, extraction time and desorption conditions (solvents, mode magnetic stir, mode ultrasonic stir, time and number of steps) assured recoveries ranging from 72 to 86%, except for phenytoin (62%). Separation was obtained using a reverse phase C18 column with UV detection (210nm). The mobile phase consisted of water: acetonitrile (78:22, v/v). The SBSE/HPLC-UV method was linear over a working range of 0.08-40.0microgmL (-1) for carbamazepine, carbamazepine-10,11-epoxide and phenobarbital and 0.125-40.0microgmL (-1) for phenytoin, the intra-assay and inter-assay precision and accuracy were studied at three concentrations (1.0, 4.0 and 20.0microgmL (-1)). The intra-assay coefficients of variation (CVs) for all compounds were less than 8.8% and all inter-CVs were less than 10%. Limits of quantification were 0.08microgmL (-1) for carbamazepine, carbamazepine-10,11-epoxide and phenobarbital and 0.125microgmL (-1) for phenytoin. No interference of the drugs normally associated with antiepileptic drugs was observed. Based on figures of merit results, the SBSE/HPLC-UV proved adequate for antiepileptic drugs analyses from therapeutic levels. This method was successfully applied to the analysis of real samples and was as effective as the LLE/HPLC-UV method.[5]

**2.6. Phenytoin (Method-I)**

Villanelli, F., et al. developed a method for quantification of phenytoin using Liquid Chromatography-Mass Spectrometry with the title "Dried blood spot assay for the quantification of phenytoin using Liquid Chromatography-Mass Spectrometry", the details of the method is described as follows: Phenytoin (PHT) is one of the most commonly used anticonvulsant drugs for the treatment of epilepsy and bipolar disorders. The large amount of plasma required by conventional methods for drug quantification makes mass spectrometry

combined with dried blood spot (DBS) sampling crucial for pediatric patients where therapeutic drug monitoring or pharmacokinetic studies may be difficult to realize. DBS represents a new convenient sampling support requiring minimally invasive blood drawing and providing long-term stability of samples and less expensive shipment and storage. The aim of this study was to develop a LC-MS/MS method for the quantification of PHT on DBS. This analytical method was validated and gave good linearity (r (2) =0.999) in the range of 0-100mg/l. LOQ and LOD were 1.0mg/l and 0.3mg/l, respectively. The drug extraction from paper was performed in a few minutes using a mixture composed of organic solvent for 80%. The recovery ranged from 85 to 90%; PHT in DBS showed to be stable at different storage temperatures for one month. A good correlation was also obtained between PHT plasma and DBS concentrations. This method is both precise and accurate and appears to be particularly suitable to monitor treatment with a simple and convenient sample collection procedure.[6]

**2.7. Phenytoin (Method-III)**

Guan, F.,et al. developed a method for Quantification of phenytoin and its metabolites in equine plasma and urine using high-performance liquid chromatography with the title "Quantification of phenytoin and its metabolites in equine plasma and urine using high-performance liquid chromatography "the details of the method is described as follows: A reliable and sensitive method for the extraction and quantification of phenytoin (5,5′-diphenylhydantoin), its major metabolite, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH) and minor metabolite, 5-(*m*-hydroxyphenyl)-5-phenylhydantoin (*m*-HPPH) in horse urine and plasma is described. The method involves the use of solid-phase extraction (SPE), liquid–liquid extraction (LLE), enzyme hydrolysis (EH) and high-performance liquid chromatography (HPLC). The minor metabolite, 5-(*m*-hydroxyphenyl)-5-phenylhydantoin (*m*-HPPH) was not present in a reliably quantifiable concentration in all samples. The new method described was successfully applied in the pharmacokinetic studies and elimination profile of phenytoin and *p*-HPPH following oral or intravenous administration in the horse.[7]

**2.8. Oxcarbazepine (Method-I)**

Paula Cristina Rezende Enéas; et al. developed a method for validation and application of an analytical method of oxcarbazepine with the title "Oxcarbazepine: validation and application of an analytical method "the details of the method is described as follows: Oxcarbazepine (OXC) is an important anticonvulsant and mood stabilizing drug. A pharmacopoeia monograph for OXC is not yet available and therefore the development and validation of a

new analytical method for quantification of this drug is essential. In the present study, a UV spectrophotometric method for the determination of OXC was developed. The various parameters, such as linearity, precision, accuracy and specificity, were studied according to International Conference on Harmonization Guidelines. Batches of 150 mg OXC capsules were prepared and analyzed using the validated UV method. The formulations were also evaluated for parameters including drug-excipient compatibility, flow ability, uniformity of weight, disintegration time, assay, uniformity of content and the amount of drug dissolved during the first hour.

Oxcarbazepine (OXC) is an anticonvulsant drug and stabilizing mood. The development and validation of analytical method for quantification of OXC are of fundamental importance because of the absence of official pharmacopoeia monographs for this drug. In this work, a UV spectrophotometric method for determination of OXC was developed. The method was validated following the linearity parameters, precision, accuracy and specificity according to the standards of the International Conference on Harmonization. OXC 150 mg Capsules were prepared and analyzed using a validated analytical method. The formulations were evaluated for drug-excipient compatibility, fluidity determining weight, disintegration time, assay, uniformity of content and amount of drug dissolved after 60 minutes.[8]

**2.9. Oxcarbazepine (Method-II)**

Jesus Antunes, et al. developed a method for Analysis of Oxcarbazepine and the 10- Hydroxycarbazepine Enantiomers with the title "Analysis of Oxcarbazepine and the 10- Hydroxycarbazepine Enantiomers in Plasma by LC- MS/MS: Application in a Pharmacokinetic Study" the details of the method is described as follows: Oxcarbazepine is a second- generation antiepileptic drug indicated as monotherapy or adjunctive therapy in the treatment of partial seizures or generalized tonic–clonic seizures in adults and children. It undergoes rapid presystemic reduction with formation of the active metabolite 10- hydroxycarbazepine (MHD), which has a chiral center at position 10, with the enantiomers (S)- (+)- and R- (−)- MHD showing similar antiepileptic effects. This study presents the development and validation of a method of sequential analysis of oxcarbazepine and MHD enantiomers in plasma using liquid chromatography with tandem mass spectrometry (LC- MS/MS). Aliquots of 100 µL of plasma were extracted with a mixture of methyl *tert*- butyl ether: dichloromethane (2:1). The separation of oxcarbazepine and the MHD enantiomers was obtained on a chiral phase Chiralcel OD- H column, using a mixture

of hexane: ethanol: isopropanol (80:15:5, v/v/v) as mobile phase at a flow rate of 1.3 mL/min with a split ratio of 1:5, and quantification was performed by LC- MS/MS. The limit of quantification was 12.5 ng oxcarbazepine and 31.25 ng of each MHD enantiomer/mL of plasma. The method was applied in the study of kinetic disposition of oxcarbazepine and the MHD enantiomers in the steady state after oral administration of 300 mg/12 h oxcarbazepine in a healthy volunteer. The maximum plasma concentration of oxcarbazepine was 1.2 µg/mL at 0.75 h. The kinetic disposition of MHD is enantioselective, with a higher proportion of the S- (+)- MHD enantiomer compared to R- (−)- MHD and an $AUC^{0-12}_{S-(+)/R-(-)}$ ratio of 5.44. *Chirality 25:897–903, 2013*. © 2013 Wiley Periodicals, Inc.[9]

**2.10. Oxcarbazepine (Method-III)**

Yang, et al. developed a method for Development and validation of an enantioselective SFC-MS/MS method for simultaneous separation and quantification of oxcarbazepine and its chiral metabolites with the title "Development and validation of an enantioselective SFC-MS/MS method for simultaneous separation and quantification of oxycarbazepine and its chiral metabolites in beagle dog plasma" the details of the method is described as follows: A rapid and sensitive assay based on supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) has been developed and validated for the determination of oxcarbazepine (OXC) and its chiral metabolite licarbazine (Lic) in beagle dog plasma using carbamazepine as internal standard. Chiral analysis in a run time of only 3 min was performed on an ACQUITY UPC (2) ™ Trefoil™ CEL2 column (3.0 × 150 mm, 2.5 µm) at 50 °C by isocratic elution with a mobile phase of supercritical carbon dioxide (purity ≥ 99.99%) and methanol (60:40, v/v) at a flow rate of 2.3 mL/min. The assay was linear over the concentration ranges 5-1000 ng/mL for OXC and 0.5-100 ng/mL for the enantiomers of Lic with corresponding lower limits of quantitation of 5 ng/mL and 0.5 ng/mL. Intra- and inter-day precisions were in the range 0.78-14.14% with accuracies in the range -10.80% to 0.42%. The method was successfully applied to a pharmacokinetic study involving a single oral administration of 16 mg/kg OXC as Trileptal (@) tablets to beagle dogs.[10]

**2.11. Oxcarbazepine (Method-IV)**

Lanckmans, et al. developed a method for Use of microbore LC-MS/MS for the quantification of oxcarbazepine and its active metabolite with the title "Use of microbore LC-MS/MS for the quantification of oxcarbazepine and its active metabolite in rat brain microdialysis samples" the details of the method is describes as follows: A microbore LC-

MS/MS method is developed and validated for the quantification of the anti-epileptic drug oxcarbazepine (OXC) and its active metabolite 10,11-dihydro-10-hydroxycarbamazepine (MHD) in rat brain microdialysates, together with the internal standard for microdialysis probe calibration, 2-methyl-5H-dibenz(b,f)azepine-5-carboxamide (m-CBZ). The benefits of gradient versus isocratic separation are shown, next to the improved sensitivity resulting from the addition of 0.1% formic acid to the mobile phase. The coupling of microdialysis with ESI-MS requires sample desalting for which column switching was applied. Using weighed regression to calculate the calibration curves (1-1000 ng/mL), the assay was validated in terms of linearity, accuracy and precision, yielding a sensitive (limit of quantification is 1 ng/mL) and selective method for quantification of OXC, MHD and m-CBZ. By applying this method, we were able to determine the extracellular concentrations of OXC and MHD during at least 4h after intraperitoneal (i.p.) administration of 10 mg/kg OXC.[11]

**2.12. Oxcarbazepine (Method-V)**

Paglia, et al. developed a method for Development and validation of a LC/MS/MS method for simultaneous quantification of oxcarbazepine with the title "Development and validation of a LC/MS/MS method for simultaneous quantification of oxcarbazepine and its main metabolites in human serum", the details of the method is described as follows: A fast, sensitive and specific LC/MS/MS method for the simultaneous analysis of oxcarbazepine (OXC), 10-hydroxycarbazepine (MHD) and trans-diol-carbazepine (DHD), in human serum, has been developed and validated. Serum drugs were extracted by C8 solid-phase cartridges (SPE) and separated in less than 3 min on a C18 reverse-phase column using an isocratic elution. A tandem mass spectrometer, as detector, was used for quantitative analysis in positive mode by a multiple reaction monitoring. Calibration curves, obtained on two ranges of concentration (0.78-50 mg/L for MHD and 0.078-5.0 mg/L for OXC and DHD), showed correlation coefficients (r) better than 0.997. Within day and between days' quality controls imprecision, as CV%, ranged from 0.3 to 4.6% and from 1.9 to 5.8%, respectively. Cyheptamide (CYE) was used as internal standard. No detectable carry-over and no relevant cross-talk and matrix effect occurred. Samples from 24 treated patients were analysed and drug serum concentrations obtained by this method are in agreement with those of other methods and also are well correlated (r=0.88) in comparison to our routine HPLC-UV method. Based on the analytical results and short run time, the method is suitable to support routine analysis of therapeutic drugs monitoring from human serum of treated patients or for pharmacokinetic studies.[12]

### 2.13. Carbamazepine, Oxcarbazepine, Eslicarbazepine Acetate

Fortuna, A., et al. developed a method for Development and validation of an HPLC-UV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate with the title "Development and validation of an HPLC-UV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate and their main metabolites in human plasma" the details of the method is described as follows: For the first time, a simple, selective and accurate high-performance liquid chromatography method with ultraviolet detection was developed and validated to quantify simultaneously three structurally related antiepileptic drugs; carbamazepine, oxcarbazepine, and the recently launched eslicarbazepine acetate and their main metabolites, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydro-carbamazepine, and licarbazepine. The method involves a solid-phase extraction and a reverse-phase C18 column with 5 cm length. The mobile phase consisting of water, methanol, and acetonitrile in the ratio 64:30:6 was selected as the best one and pumped at 1 mL/min at 40 degrees C. The use of this recent column and an aqueous mobile phase instead of buffers gives several advantages over the method herein developed; namely the fact that the chromatographic analysis takes only 9 min. The method was validated according to the guidelines of the Food and Drug Administration, showing to be accurate (bias within +/-12%), precise (coefficient variation <9%), selective and linear (r(2) > 0.997) over the concentration range of 0.05-30 microg/mL for carbamazepine; 0.05-20 microg/mL for oxcarbazepine; 0.15-4 microg/mL for eslicarbazepine acetate; 0.1-30 microg/mL for carbamazepine-10,11-epoxide; 0.1-10 microg/mL for 10,11-trans-dihydroxy-10,11-dihydro-carbamazepine, and 0.1-60 microg/mL for licarbazepine. It was also shown that this method can adequately be used for the therapeutic drug monitoring of the considered antiepileptic drugs, carbamazepine, oxcarbazepine, eslicarazepine acetate, and their metabolites.[13]

### 2.14. Carbamazepine, Oxcarbazepine (Method-I)

Ferreira, A., et al. developed a method for HPLC–DAD Method for the quantification and their Active Metabolites in HepaRG Cell Culture Samples with the title "Samples "the details of the method is described as follows: A new, sensitive and fast high-performance liquid chromatography–diode-array detection assay is herein reported, for the first time, to simultaneously quantify carbamazepine (CBZ), oxcarbazepine (OXC), and the active metabolites carbamazepine-10,11-epoxide (CBZ-E) and licarbazepine (LIC) in HepaRG cell culture medium samples. Chromatographic separation of analytes (CBZ, CBZ-E, OXC,

LIC) and internal standard (IS) was achieved in less than 15 min on a $C_{18}$-column, at 35 °C, using a mobile phase composed of water/methanol/acetonitrile (69:25:6 v/v/v) pumped at 1 mL $min^{-1}$. The analytes and IS were detected at 215 nm. The method proved to be selective, accurate (*bias* ± 14.6%), precise (coefficient of variation ≤13.1%) and linear ($r^2 \geq 0.9901$) over the concentration ranges of 0.1–15 μg $mL^{-1}$ for CBZ; 0.1–5 μg $mL^{-1}$ for CBZ-E and OXC; and 0.1–40 μg $mL^{-1}$ for LIC. Furthermore, the absolute recovery of the analytes ranged from 64.5 to 96.9% and their stability was demonstrated in the studied conditions. This validated HPLC assay will be a suitable tool to support future in vitro metabolism profiling, drug interaction and other pharmacokinetic-based studies in HepaRG cells involving these antiepileptic drugs (CBZ and OXC) and their main metabolites.[14]

### 2.15. Carbamazepine, Oxcarbazepine (Method-II)

Lionetto, L., et al. developed a method for High-Performance Liquid Chromatography-Tandem Mass Spectrometry Method for Simultaneous quantification and Their Main Metabolites in Human Serum with the title "High-Performance Liquid Chromatography-Tandem Mass Spectrometry Method for Simultaneous quantification of Carbamazepine, oxycarbazepine and Their Main Metabolites in Human Serum" details of the method is described as follows: Antiepileptic drug therapeutic regimens often need to be adjusted individually on the basis of serum assays. We aimed to develop a quantitative, fast, and sensitive liquid chromatography-tandem mass spectrometry method to simultaneously analyze carbamazepine, oxcarbazepine, and the 10-11 epoxide carbamazepine and 10-hydroxy carbazepine (mono-hydroxy derivative, 10,11-Dihydro-10-hydroxycarbamazepine) metabolites, in human serum. Serum samples were deproteinized by acetonitrile spiked with dansyl-norvaline as internal standard. Compounds were separated on a reversed-phase high-performance liquid chromatography over a total run time of 10 minutes. Serum concentrations were then measured by means of a triple quadrupole tandem mass spectrometer, set up in positive mode and multiple reaction monitoring. Calibration curves (0.08-50 mcg/mL for carbamazepine and 10,11-dihydro-10-hydroxycarbamazepine; 0.03-20 mcg/mL for oxcarbazepine and epoxide carbamazepine) were linear, with a mean correlation coefficient >0.999. Both the intra- and interassay imprecision and inaccuracy were within 10%. The absolute recovery ranged from 98% to 103% for all analytes. The method requires minimal sample preparation. Volume of the sample is lower and run time shorter than required by previous published liquid chromatography-tandem mass spectrometry methods. Results are accurate. The method seems, therefore, to be reliable and

economically suitable for routine analysis of antiepileptic drugs monitoring in clinical settings.[15]

### 2.16. Ethosuximide

Sghendo, L., et al. developed a method for sensitive gas chromatographic/mass spectrometric method for the resolution and quantification of ethosuximide enantiomers in biological fluids with the title "A sensitive gas chromatographic/mass spectrometric method for the resolution and quantification of Ethosuximide enantiomers in biological fluids" details of the method is described as follows: A modified specific, sensitive and reproducible chiral gas chromatographic (GC) method for the resolution and quantification of Ethosuximide enantiomers in urine and plasma was developed. The samples were extracted by liquid-liquid extraction, using diethylether and the enantiomers were separated and quantified on a chiral gas chromatographic column (25QC2 / CYDEX- beta 0.25). The method involved the use of GC/MS instrumentation for the acquisition of data in the electron impact selective-ion monitoring mode, collecting ions characteristic of both Ethosuximide and alpha, alpha - dimethyl - beta - methylsuccinimide, the internal standard and of mass-to-charge ratio (m/z) exactly equal to 55 and 70 units. The limit of quantitation of the method was 2.5 microg/ml for both urine and plasma with both enantiomers. The method proved to be linear, precise and reproducible in the 5-300 microg/ml concentration range for urine samples and in the 10-250 microg/ml concentration range for plasma samples. Future research work envisaged the application of this method in pharmacokinetic and pharmacodynamics studies.[16]

### 2.17. Asenapine and valproic acid

Ambavaram Vijaya Bhaskar Reddy, et al. developed a method for Simultaneous determination of asenapine and valproic acid using LC-MS/MS with the title "Simultaneous determination of asenapine and valproic acid in human plasma using LC-MS/MS: Application of the method to support pharmacokinetic study" details of this method is described as follows: Combination of asenapine with valproic acid received regulatory approval for acute treatment of schizophrenia and maniac episodes of bipolar disorders. A simple LC-MS/MS method was developed and validated for simultaneous quantification of asenapine and valproic acid in human plasma. Internal standards were added to 300 µL of plasma sample prior to liquid-liquid extraction using methyl tertiary butyl ether (MTBE). Chromatographic separation was achieved on Phenomenex C18 column (50 mm×4.6 mm, 5 µm) in isocratic mode at 40 °C. The mobile phase used was 10 mm ammonium formate-

acetonitrile (5:95, v/v) at a constant flow rate of 0.8 mL/min monitored on triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) mode. The injection volume used for LC-MS/MS analysis was 15 µL and the run time was 2.5 min. These low run time and small injection volume suggest the high efficiency of the proposed method. The method was validated over the concentration range of 0.1-10.02 ng/mL and 10-20,000 ng/mL for asenapine and valproicacidrespectively. The method recoveries of asenapine (81.33%), valproic acid (81.70%), gliclazide (78.45%) and benzoic acid (79.73) from spiked plasma samples were consistent and reproducible. The application of this method was demonstrated by a pharmacokinetic study in 8 healthy male volunteers with 5 mg asenapine and 250 mg valproic acid administration.[17]

### 2.18. Valproic acid

Cheng, H., et al. developed a method forQuantification of valproic acid and its metabolite using HPLC-MS/MS with the title "Quantification of valproic acid and its metabolite 2-propyl-4-pentenoic acid in human plasma using HPLC-MS/MS"details of this method is described as follows: : A specific and sensitive HPLC-MS/MS method for the quantitative determination of valproic acid (VPA) and its metabolite, 2-propyl-4-pentenoic acid in human plasma has been developed, using VPA-d15 as the internal standard. The method was based on pre-column derivatization using 4-dimethylaminobenzylamine dihydrochloride. The derivatives were separated with a gradient elution and quantified by positive electrospray ionization with multiple reaction monitoring. The assay provides routine quantification limits of 200 ng/mL for VPA and 20 ng/mL for 4-ene VPA with within- and between-day coefficients of variation of <10%. This method has been applied to the analysis of plasma samples obtained from patients treated with this drug.[18]

### 2.19. Clonazepam and Paroxetine hydrochloride

Purvi Shah, et al. developed a method for Development and validation of an HPTLC method for the simultaneous estimation using a DOE approach with the title "Development and validation of an HPTLC method for the simultaneous estimation of Clonazepam and Paroxetine hydrochloride using a DOE approach" details of this method is described as follows: The present study examines simultaneous multiple response optimization using Derringer's desirability function for the development of an HPTLC method to detect Clonazepam and Paroxetine hydrochloride in pharmaceutical dosage form. Central composite design (CCD) was used to optimize the chromatographic conditions for HPTLC. The

independent variables used for the optimization were the n-butanol content in the mobile phase, the chamber saturation time and the distance travelled. HPTLC separation was performed on aluminium plates pre-coated with silica gel 60 F254 as the stationary phase using n-butanol& 58; glacial acetic acid& 58; water (9&58;2&58;0.5% v/v/v) as the mobile phase. Quantification was achieved based on a densitometric analysis of Clonazepam and Paroxetine hydrochloride over the concentration range of 40–240 ng/band and 300–1800 ng/band, respectively, at 288 nm. The method yielded compact and well-resolved bands at Rf of 0.77 ± 0.02 and 0.34 ± 0.02 for Clonazepam and Paroxetine hydrochloride, respectively. The linear regression analysis for the calibration plots produced r2 &61; 0.9958 and r2 &61; 0.9989 for Clonazepam and Paroxetine hydrochloride, respectively. The precision, accuracy, robustness, specificity, limit of detection and limit of quantitation of the method were validated according to the ICH guidelines. The factors evaluated in the robustness test were determined to have an insignificant effect on the selected responses. The results indicate that the method is suitable for the routine quality control testing of marketed tablet formulations.[19]

**2.20. Clonazepam (Method-I)**

Favreto, et al. developed a method for Development and Validation of an Ultra-Performance Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry Bioanalytical Method in Human Plasma with the title "Development and Validation of an Ultra-Performance Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry Bioanalytical Method for Quantifying Clonazepam in Human Plasma"details of this method is described as follows: A sensitive, selective, and rapid ultra-performance LC (UPLC)/MS/MS method was validated for the confirmation and quantification of clonazepam in human plasma. The analyte was extracted from human plasma with diethyl ether, reaching an average recovery of 64.02 and 66.48% for clonazepam and the internal standard, respectively. The separation was performed on a Waters ACQUITY UPLC™ BEH C18 column (50 × 2.1 mm id, 1.7 µm particle size) with gradient elution at a flow rate of 0.25 mL/min using a 0.5% formic acid solution (mobile phase A) and acetonitrile–methanol–formic acid (75 + 25 + 0.5, v/v/v; mobile phase B). Detection was performed on a triple-quadruple tandem mass spectrometer in the multiple reaction monitoring mode via electrospray ionization. Linear calibration curves were obtained in the concentration range of 0.3–50.0 ng/mL, with an LOQ of 0.3 ng/mL. The intraday and interday precision (CV) values were below 10%, and accuracy (relative error) ranged from −2.6 to 6.6% at all QC levels.

The suggested method was successfully applied for the determination of clonazepam in human plasma in a bioequivalence study.[20]

### 2.21. Clonazepam (Method-II)

Gu, J., et al. developed a method for LC–MS Method for the Quantification of Clonazepam in Rat Plasma with the title "LC–MS Method for the Quantification of Clonazepam in Rat Plasma" details of this method are described as follows: A sensitive and specific high-performance liquid chromatography–tandem mass spectrometry method has been developed and validated for the determination of clonazepam in rat plasma. Clonazepam and internal standard diazepam were extracted from plasma samples by a single-step protein precipitation. The chromatographic separation was performed on a Dikma ODS-C18 reversed-phase column at 40 °C. The mobile phase composed of a premix of solvent A (0.1% formic acid–4 mM ammonium acetate–water)–solvent B (acetonitrile) (13:87, *v*/*v)* at a flow-rate of 0.7 mL $min^{-1}$. Positive electrospray ionization was utilized as the ionization source. Clonazepam and the internal standard were determined using multiple reaction monitoring of precursor → product ion transitions at *m*/*z* 316.0 → 270.0 and *m*/*z* 285.1 → 193.2, respectively. The lower limit of quantification was 0.25 ng $mL^{-1}$ using 50 µL plasma samples and the linear calibration range was from 0.25 to 128 ng $mL^{-1}$. The within- and between-batch RSDs were lower than 15% and the relative recoveries of clonazepam ranged from 97.4 to 104.7%. The mean extraction recoveries of clonazepam and IS were 79.7 and 77.6%, respectively. The method has been successfully applied to the pharmacokinetic studies in rat after oral administration of clonazepam.[21]

### 2.22. Diazepam (Method-I)

PatríciaRijo, et al. developed a method with the title "A didactic approach for quantification of diazepam tablets by UV spectrophotometry" details of this method is described as follows: The present paper describes a simple laboratory procedure for the quantification of diazepam, a central nervous system depressant benzodiazepine, by ultraviolet spectroscopy. Standard solutions of diazepam, prepared from a stock solution, were used to construct a calibration curve. Commercial tablets of diazepam were then analysed and the results obtained were in accordance with the expected percentage range of the manufacturer's claim and with the specifications of the Portuguese Pharmacopeia.[22]

### 2.23. Diazepam (Method-II)

ĐorđevićSnežana et al. developed a method with the title "Determination of diazepam and its metabolites in serum by the use of liquid chromatography: Mass spectrometry method" details of this method is described as follows: Diazepam is a benzodiazepine anxyolitic. Metabolism of diazepam takes place in liver which generates pharmacologically active metabolites N-desmethyldiazepam, temazepam and oxazepam. The aim of this study was to develop and validate the method of liquid chromatographymass spectrometry (LC-MS) for separation and determination of diazepam and its active metabolites in the serum of rats samples after i.p. application of diazepam in a dose of 10 mg/kg. Methods. The serum samples taken from Wistar rats, were used in LC-MS analysis after the application of 10 mg/kg of diazepam i.p. Results. After alkaline extraction from the serum samples with diethylether and separation on a C18 reversed-phase column by using mobile phase methanolglacial acetic acid-water (50:1:49 v/v), diazepam and its metabolites were quantified. Determination was performed in a selective ion monitoring (SIM) mode, thereby the other exogenous and endogenous compounds did not interfere with this assay. Diazepam, N-desmethyldiazepam, oxazepam and temazepam were eluted in 14 minutes. The standard curve was linear in the range from 10-2 000 ng/ml. The limits of detection for diazepam, N-desmethyldiazepam, oxazepam and temazepam were 4.37, 3.13, 4.38 and 7.31 ng/ml, respectively. The limits of quantitation for diazepam, Ndesmethyldiazepam, oxazepam and temazepam were 14.58, 10.41, 14.59 and 24.36 ng/ml, respectively. The described LC-MS is a simple, sensitive, specific and accurate method and could be used for routine identification and quantification of small concentrations of diazepam and its metabolites in biological fluids.[23]

### 2.24. Diazepam and Dexamethasone

Wenwen Gong et al. developed the method for Simultaneous Quantification of Diazepam and Dexamethasone in Plasma by High-Performance Liquid Chromatography with Tandem Mass Spectrometry with the title "Simultaneous Quantification of Diazepam and Dexamethasone in Plasma by High-Performance Liquid Chromatography with Tandem Mass Spectrometry and Its Application to a Pharmacokinetic Comparison between Normoxic and Hypoxic Rats" details of this method are described below as follows: In order to investigate the pharmacokinetics of a combination of diazepam and dexamethasone under hypoxic conditions, a novel, sensitive and specific liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of diazepam and

dexamethasone in rat plasma was developed and validated. The chromatographic separation of analytes was successfully achieved on an XTerra® MS C18 column using a gradient elution of methanol and water containing 0.1% formic acid at a flow rate of 0.5 mL/min. This method demonstrated good linearity and no endogenous material interferences. The linear ranges were 1.0–100 ng/mL for diazepam and 2.0–200 ng/mL for dexamethasone. The intra- and inter-day precision for the two compounds in plasma were lower than 10.0%, and the accuracy was between −7.9% and 11.5%. Our method was then successfully applied in a pharmacokinetic comparison between normoxic and hypoxic rats. The results indicated that there were significant differences in the main pharmacokinetics parameters of diazepam and dexamethasone between normoxic and hypoxic rats. The results provide the important and valuable information for discovering and developing novel anti-hypoxia drug combinations, as well as a better understanding of the safety and efficacy of these drugs.[24]

### 2.25. Nitrazepam and Lorazepam

Molaei, et al. developed a method for Surfactant assisted dispersive liquid–liquid microextractionwith the title "Surfactant assisted dispersive liquid–liquid microextraction of nitrazepam and lorazepam from plasma and urine samples followed by high performance liquid chromatography with UV analysis" details of this method are described as follows: Surfactant assisted liquid–liquid microextraction followed by high performance liquid chromatography with UV detection has been developed for the simultaneous preconcentration and determination of lorazepam and nitrazepam in biological fluids. In this study, an ionic surfactant (cetyltrimethyl ammonium bromide) was used as an emulsifier. The predominant parameters affecting extraction efficiency such as the type and volume of extraction solvent, the type and concentration of surfactant, sample pH, and the concentration of salt added to the sample were investigated and opted. Under the optimum conditions (extraction solvent and its volume, 1-octanol, 70 μL; surfactant and its concentration, 1 mL of ultra pure water containing 2 mmol $L^{-1}$ cetyltrimethyl ammonium bromide; sample pH = 9 and salt content of 10% NaCl w/v), the preconcentration factors were obtained in the range of 202–241 and 246–265 for nitrazepam and lorazepam, respectively. The limits of quantification for both drugs were 5 μg $L^{-1}$ in water sample and 10 μg $L^{-1}$ in biological fluids with $R^2$ values higher than 0.993. The suitability of the proposed method was successfully confirmed by the extraction and determination of the target drugs in human urine and plasma samples in the range of microgram per liter.[25]

### 2.26. Gabapentin

Ramakrishna, N. V., et al. developed a method for Rapid quantification by liquid chromatography/tandem mass spectrometry with the title "Rapid quantification of gabapentin in human plasma by liquid chromatography/tandem mass spectrometry" details of this method is described as follows: A simple, sensitive and rapid liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of gabapentin, a new antiepileptic drug, in human plasma using its structural analogue, 1,1-cyclohexane diacetic acidmonoamide (CAM) as internal standard. The method involved a simple protein precipitation by means of acetonitrile followed by a rapid isocratic elution with 10mM ammonium formate buffer/acetonitrile (20/80, v/v, pH 3.0) on Waters Symmetry C(18 reversed phase chromatographic column and analyzed by mass spectrometry in the multiple reaction monitoring mode. The precursor to product ion transitions of m/z 172-->154 and m/z 200-->182 were used to measure the analyte and the IS, respectively. The assay exhibited a linear dynamic range of 40-10000 ng/mL for gabapentin in human plasma. The limit of detection and lower limit of quantification in human plasma were 10 and 40 ng/mL, respectively. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. A run time of 2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies.[26]

### 2.27. Levetiracetam and Gabapentin

Juenke, et al. developed a method for Simultaneous quantification by Ultra-Pressure Liquid Chromatography Coupled with Tandem Mass Spectrometry Detection with the title ":Simultaneous quantification of Levetiracetam and gabapentin in Plasma by Ultra-Pressure Liquid Chromatography Coupled with Tandem Mass Spectrometry Detection "details of this method are described below as follows: Gabapentin (Neurontin) and levetiracetam (Keppra) are anticonvulsants with novel structures and suggested therapeutic ranges of 2-10 mg/L and 6-20 mg/L, respectively. Gabapentin is also used extensively to manage neuropathic pain, and for this indication, wherein higher doses are prescribed, plasma concentrations of 15-30 mg/L are typical. Here, we describe a simple rapid assay to support therapeutic drug monitoring of gabapentin and levetiracetam in plasma by ultra-pressure liquid chromatography couples to tandem mass spectrometry (UPLC-MS/MS) detection. After the addition of internal standard and protein precipitation of patient plasma with methanol:

acetonitrile in a 50:50 ratio, 1 µL of supernatant sample is injected onto an Acquity UPLC HSS T3, 1.8 µm, 2.1 × 50 mm (Waters) column. Elution occurs using a linear gradient of acetonitrile and water, each having 0.1% formic acid added. The column is eluted into a Waters Acquity UPLC TQD, operating in a positive mode to detect gabapentin at transition 172.18 > 154.11, levetiracetam at 171.11 > 126, and internal standard (3-amino-2-naphthoic acid) at 188.06 > 170. Secondary transitions for each analyte are also monitored for gabapentin at 172.18 > 137.06, levetiracetam at 171.11 > 154, and internal standard at 188.06 > 115. Runtime is 1.5 minutes per injection with baseline resolved chromatographic separation. The analytical measurement ranges were 1-150 mg/L for gabapentin and for levetiracetam. Intra-assay imprecision by the coefficient of variance (CV) was less than 8% and interassay CV was less than 5% for both analytes, at 4 different concentrations. Results obtained from patient samples were compared with results generated by established high-performance liquid chromatography-UV methods with the following regression statistics: y = 1.12x - 0.77, r = 0.996, Sy, x = 0.89, and n = 29 for gabapentin and y = 0.991x + 0.70, r = 0.997, Sy, x = 2.24, and n = 30 for levetiracetam. No analytical interferences were identified. In summary, a simple reliable UPLC-MS/MS method was developed and validated for routine clinical monitoring of gabapentin and levetiracetam.[27]

**2.28. Gabapentin, Pregabalin, and Vigabatrin**

Chahbouni, et al. developed the methods for Rapid quantification by Ultra performance Liquid Chromatography With Mass-Spectrometric Detection with the title "Rapid quantification of gabapentin, Pregabalin, and Vigabatrin in Human Serum by Ultra performance Liquid Chromatography With Mass-Spectrometric Detection" details of this method is described as follows: Gabapentin (GBP), pregabalin (PRG), and vigabatrin (VIG) are used for the prevention and treatment of epileptic seizures. The developed method was applied to samples from subjects participating in a pharmacokinetic study of GBP. Sample pretreatment consisted of adding 20 µL of trichloroacetic acid (30%; vol/vol) and 200 µL of GBP-d4 in acetonitrile as an internal standard to 20 µL of serum. Chromatographic separation was performed on an Acquity separation module using a Kinetex RP18 column. The aqueous and organic mobile phases were 2 mM ammonium acetate supplemented with 0.1% formic acid in water and acetonitrile, respectively. The detection by a tandem quadrupole mass spectrometer, operating in the positive mode using multiple reaction monitoring, was completed within 2 minutes. The method was linear over the range of 0.03-25 mg/L for GBP, 0.03-25 mg/L for PRG, and 0.06-50 mg/L for VIG. The between- and within-run accuracies

ranged from 90% to 107%. The between- and within-run imprecisions of the method were <10%. Stability data show no significant decrease of the analytes. A relative matrix effect of -1%, 0.2%, and -5% was determined for GBP, PRG, and VIG, respectively. A simple and sensitive ultraperformance liquid chromatography-tandem mass spectrometry method was developed and validated for the simultaneous quantification of GBP, PRG, and VIG in human serum. The reported method provided the necessary linearity, precision, and accuracy to allow the determination of GBP, PRG, and VIG for therapeutic drug monitoring and clinical research purposes.[28]

**2.29. Pregabalin**

Lakshmi Narayana Chennuru, et al. developed a method for separation of pregabalin with the title "Direct Separation of Pregabalin Enantiomers Using a Zwitterionic Chiral Selector by High Performance Liquid Chromatography Coupled to Mass Spectrometry and Ultraviolet Detection" details of this method are as follows: The chromatographic resolution of pregabalin enantiomers has been often achieved by derivatization of the molecule, in order to reach enough sensitivity at low concentrations of the minor enantiomer present in the active principle. In the present article, the development and optimization of two liquid chromatographic methods are presented for the direct resolution of pregabalin enantiomers on a chiral stationary phase (CSP) containing a zwitterionic selector derived from cinchona alkaloid and sulfonic acid (CHIRALPAK ZWIX). The key parameters for the separation as well as the compatibility of chromatographic conditions with different detection modes (ultraviolet and mass spectrometry) were investigated. The resulting methods were found to be selective, of high performance and low limits of detection (2 µg/mL by UV and 1 ng/mL by MS, respectively) and quantification (6 µg/mL by UV and 5 ng/mL by MS, respectively) for the minor enantiomer which is considered as a chiral impurity.[29]

**2.30. Topiramate**

Koba, et al. developed a method for Application of HPTLC and LC-MS Methods with the title "Application of HPTLC and LC-MS Methods for Determination of Topiramate in Pharmaceutical Formulations" details of this method are described as follows: Methods for determination of topiramate in pharmaceutical formulation by high-performance thin-layer chromatography (HPTLC) UV-densitometry and liquid chromatography-mass spectrometry (LC-MS) have been developed. HPTLC method as recommended by United States Pharmacopeia was performed using the silica plates, mobile phase composed of

benzene:ethanol (5:2, v/v) and densitometric detection at 340 nm after topiramate visualization with the use of chemical reagent. Moreover, quantification was achieved in the concentration range of 0.25-4.0 µg/spot and with adequate precision (RSD=4.16%) and recovery (104.47%) using non-linear calibration curve by fitting to y=a+blnx. LC-MS method was performed using Zorbax SB-C18 column and isocratic elution mode with a mobile phase composed of acetonitrile: water (85:15, v/v) with 0.1% (v/v) formic acid at a flow rate 0.5 ml/min. Moreover, the single quadrupole mass spectrometer employing ESI interface operated in the negative ion mode was used to quantify the topiramate at m/z=338.1. Proposed LC-MS method provided good results of precision (RSD=2.05%) and recovery (99.53%) in the concentration range of 0.25-10.0 µg/ml using linear y=a+bx regression analysis. Additionally, no interferences were found from tablet excipients at the selected wavelength, mass-to-charge ratio and assay procedures. The developed methods found to be sufficiently precise and reproducible for established conditions and after validation may be used for routine quality control of topiramate in pharmaceutical formulations.[30]

**2.31. Levetiracetam (Method-I)**

HU, Lan-lan, et al. developed a method for Quantification by ultra-performance liquid chromatography-tandem mass spectrometry with the title "Quantification of levetiracetam in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry and its application in a pharmacokinetic study" details of this method is as follows: Objective: To develop a specific and rapid method for the determination of levetiracetam in plasma using ultra performance liquid chromatography-tandem mass spectrometry(UPLC-MS/MS), and study pharmacokinetics of levetiracetam. Methods: A plasma aliquot of 0.2 mL was deproteinized by addition of 280 µL methanol which contained 3-amino-2-naphthoic acid as an internal standard. After centrifugation, 3 µL of supernatant was injected into the UPLC-system. Compounds were separated on an Acquity UPLC BEH $C_{18}$(2.1 mm×50 mm, 1.7 µm) column using gradient elution with mobile phase acetonitrile and 0.01 mol·$L^{-1}$ ammonium formate (pH=3.6) with a flow rate of 0.3 mL·$min^{-1}$ and a total runtime of 4.0 min. Levetiracetam and the internal standard were detected using positive ion electrospray ionization followed by tandem mass spectrometry(ESI-MS/MS). Results: The assay allowed quantification of levetiracetam plasma concentrations in the range of 0.5-128 µg·$mL^{-1}$. Intra-assay accuracy was below 13.11% and inter-assay precision was less than 11.7%. Matrix effects were minor, and the recovery of levetiracetam was between 95.9% and 106.8%. The maximum concentration($C_{max}$) were 18.23 µg·$mL^{-1}$, and the time to reach this

concentration($T_{max}$) was 1.31 h.Conclusion: The developed method required less sample volume of plasma. The method has been applied in a clinical pharmacokinetic study in which adult/neonates received levetiracetam for the treatment of seizures.[31]

**2.32. Levetiracetam (Method-I)**

Matar, K. M. at. Al developed a method for Quantification by liquid chromatography-tandem mass spectrometry with the title "Quantification of levetiracetam in human plasma by liquid chromatography-tandem mass spectrometry: Application to therapeutic drug monitoring" details of this method is as follows: A rapid, selective, reliable, precise, accurate, and reproducible tandem mass spectrometric (MS-MS) method for the quantification of levetiracetam (LEV) in human plasma using adenosine as an internal standard (IS) has been developed and validated. The drug and IS were extracted by solid phase extraction (SPE) technique and analyzed on Symmetry((R)) C(18) column (5 microm, 3.9 mm x 50 mm) using a mobile phase of methanol-water-formic acid (97:03:0.25, v/v/v) at a flow rate of 0.2 ml/min. Quantitation was achieved using a positive electrospray ionization (ESI+) interface employing multiple reaction monitoring (MRM) mode at MRM transitions m/z 171>126 and m/z 268>136 for LEV and IS, respectively. The method was validated over the concentration range of 1.0-40 microg/ml (r>0.99) with a limit of quantification of 1.0 microg/ml (R.S.D.%; 4.1 and Bias%; -9.0 to + 11.0%). Intra- and inter-run precision of LEV assay at three concentrations ranged from 0.6 to 8.9% with accuracy (bias) varied from -4.0 to 8.6% indicating good precision and accuracy. Analytical recoveries of LEV and IS from spiked human plasma were in the range of 91.7-93.4% and 80.2-84.1%, respectively. Stability of LEV in human plasma samples at different conditions showed that the drug was stable under the studied conditions. Matrix effect study showed a lack of matrix effect on mass ions of LEV and IS. The described method compared well with the commercial HPLC-UV method of Chromsystem (r(2)=0.99). The suitability of the developed method for therapeutic drug monitoring was demonstrated by measuring LEV in human plasma samples of epileptic patients treated with LEV.[32]

**2.33. Lacosamide**

Chhalotiya Usmangani K., et al. developed a method for stability with the topic "Stability-indicating liquid chromatographic method for quantification of new anti-epileptic drug lacosamide in bulk and pharmaceutical formulation" details of thos method is as follows: An isocratic stability indicating reversed-phase liquid chromatographic determination was

developed for the quantitative determination of lacosamide in the pharmaceutical dosage form. A Hypersil C-18, 4.5µm column with mobile phase containing acetonitrile-water (20:80, v/v) was used. The flow rate was 1.0 mL min-1 and effluents were monitored at 258 nm. The retention time of lacosamide was 8.9 min. The method was found to be linear in the concentration range of 5-100 µg/ml and the recovery was found to be in the range of 99.15 - 100.09%. The limit of detection and limit of quantification were found to be 2 µg/ml and 5 µg/ml, respectively. Lacosamide stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation and dry heat degradation. The drug was found to be stable to the dry heat and acidic condition attempted. The proposed method was validated and successfully applied to the estimation of lacosamide in tablet dosage forms.[33]

**2.34. Anti epileptic drugs and two active metabolites**

Karinen, et al. developed a method for Determination by reversed phase UPLC-MS/MS with the title "Determination of a selection of anti-epileptic drugs and two active metabolites in whole blood by reversed phase UPLC-MS/MS and some examples of application of the method in forensic toxicology cases" details of this method is as follows: Quantitative determination of anti-epileptic drug concentrations is of great importance in forensic toxicology cases. Although the drugs are not usually abused, they are important post-mortem cases where the question of both lack of compliance and accidental or deliberate poisoning might be raised. In addition, these drugs can be relevant for driving under the influence cases. A reversed phase ultra-performance liquid chromatography-tandem mass spectrometry method has been developed for the quantitative analysis of the anti-epileptic compounds carbamazepine, carbamazepine-10,11-epoxide, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, 10-OH-carbazepine, phenobarbital, phenytoin, pregabalin, and topiramate in whole blood, using 0.1 mL sample volume with methaqualone as internal standard. Sample preparation was a simple protein precipitation with acetonitrile and methanol. The diluted supernatant was directly injected into the chromatographic system. Separation was performed on an Acquity UPLC® BEH Phenyl column with gradient elution and a mildly alkaline mobile phase. The mass spectrometric detection was performed in positive ion mode, except for phenobarbital, and multiple reaction monitoring was used for drug quantification. The limits of quantification for the different anti-epileptic drugs varied from 0.064 to 1.26 mg/L in blood, within-day and day-to-day relative standard deviations from 2.2 to 14.7% except for phenobarbital. Between day variation for phenobarbital was 20.4% at the concentration level of 3.5 mg/L. The biases for all compounds were within ±17.5%. The recoveries ranged

between 85 and 120%. The corrected matrix effects were 88–106% and 84–110% in ante-mortem and post-mortem whole blood samples, respectively. Copyright © 2014 John Wiley & Sons, Ltd. A fully validated reversed phase ultra-performance liquid chromatography-tandem mass spectrometry method for the quantitative analysis of the antiepileptic compounds carbamazepine, carbamazepine-10,11-epoxide, gabapen-tin, lamotrigine, levetiracetam, oxcarbazepine, 10-OH-carbazepine, phenobarbital, phenytoin, pregabalin and topiramate in whole blood, using 0.1 mL samples, with methaqualone as internal standard, is presented. Sample preparation is a protein precipitation with a mixture of ice-cold acetonitrile and methanol. The mass spectrometric analysis is performed in positive ion mode, except for phenobarbital, and multiple reaction monitoring (MRM).[34]

## 3. CONCLUSION

In the analysis of of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine, sample preparation consisted of a deproteinization step with methanol followed by a solid- phase extraction procedure. In the estimation of Phenytoin**,** the sample preparation involves a rapid and simple procedure based on liquid-liquid extraction. A sensitive and reproducible stir bar-sorptive extraction and high-performance liquid chromatography-UV detection (SBSE/HPLC-UV) method for therapeutic drug monitoring of carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbital in plasma samples is reported in literature, In the qualitative estimation of Phenytoin, the method involves the use of solid-phase extraction (SPE), liquid–liquid extraction (LLE), enzyme hydrolysis (EH) and high-performance liquid chromatography (HPLC). In the estimation of Oxcarbazepine Serum drugs were extracted by C8 solid-phase cartridges (SPE) and separated in less than 3 min on a C18 reverse-phase column using an isocratic elution. In the simultaneous estimation of carbamazepine, oxcarbazepine, eslicarbazepine acetate, the method involves a solid-phase extraction and a reverse-phase C18 column with 5 cm length. In the quantitative estimation of asenapine and valproic acid, internal standards were added to 300 μL of plasma sample prior to liquid-liquid extraction using methyl tertiary butyl ether. In the estimation of Levetiracetam, the drug and IS were extracted by solid phase extraction (SPE) technique. Altogether it is said to be above reported methods were fully validated can be used for routine analysis.

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