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A REVIEW OF ANALYTICAL METHODS ON LAMOTRIGINE

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ABSTRACT

Lamotrigine is an anti-epileptic drug belongs to phenyl triazine class. It is used in the treatment of both epilepsy and as a mood stabilizer in bipolar disorder. In this article different analytical methods such as spectrophotometry, chromatography methods like HPLC (high performance liquid chromatography), liquid chromatography-tandem mass spectrometry etc, are used for the pharmaceutical evaluation of lamotrigine for anti-epileptic activity.

Keywords: Lamotrigine, RP-HPLC, UV, flow rate

INTRODUCTION

Lamotrigine is used in the treatment of epilepsy. Lamotrigine chemically known as 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine. It is used for the treatment patients with reterofactory partial seizure. Lamotrigine mechanism of action is not clear, but it appears to be acts by inhibiting the presynaptic voltage-sensitive sodium channels and excitatory neurotransmitter release. Chemical formula and molecular weight are C9H7Cl2N5 and 256.091 g/mol. Lamotrigine is metabolized by liver (mostly UGT 1A4-mediated) and excreted by urine (65%) and feces (2%), its bioavailability is 98% and its half-life is 29 hours. Estimation of the mean apparent volume of distribution of lamotrigine flowing oral administration ranges from 0.9 to 1.3 L/kg. Common side effects for the lamotrigine are nausea, sleepiness, headache, vomiting, trouble with coordination and rash and serious are lack of RBCs, increase risk of suicide, Stevens-Johnson syndrome and allergic reactions. It is considered as a first-

line drug for primary generalized tonic-clonic seizures. A 2020 review on the use of lamotrigine as add-on therapy for drug resistant generalized tonic-clonic seizures. Due to pharmacological activity of lamotrigine, it is formulated and analyzed in different analytical techniques **[1]**.

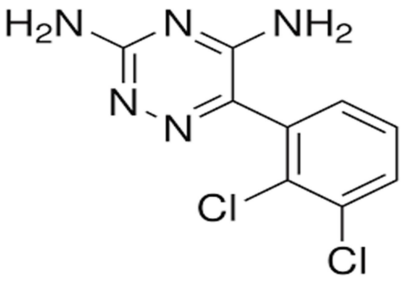

Figure 1: Lamotrigine

Table 1

S. No.	DESCRIPTION	PARAMETERS
1.	Lamotrigine in bulk and dosage form by UV-Spectrophotometry [2].	Solvent: 0.1M NaOH Wave length: 307 nm Linearity: 5 to 50 µg/ ml Correlation coefficient: 0.99768 Percent RSD: 0.130
2.	Simultaneous Analysis of Lamotrigine, Oxcarbazepine, 10-Hydroxycarbazepine, and Zonisamide by HPLC–UV and a Rapid GC Method Using a Nitrogen-Phosphorus Detector for Levetiracetam[3]	Column: supelcosil LC-18 column (25cm× 4.6 mm i.d, 5µM silica particles; Supelcosil, Bellefonte, PA) Mobile phase: 3 mM potassium phosphate buffer (adjusted to pH 3.7 with 5% phosphoric acid): acetonitrile (65:35) Floe rate: 1.2 ml/min Temperature: 22^0 C Detection wavelength: Oxcarbazepine and 10-hydroxycarbazepine at 230nm Lamotrigine and zonisamide at 270nm
3.	Development and Validation of HLPC Method for the Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations [4]	Column:symmetry C8 (4.6mm ID × 150 mm; 3.5 µm, Make: X Terra) Flow rate: 0.7 ml/min Mobile phase: acetonitrile: potassium dihydrogen phosphate (buffer pH 7.0) ratio of 60:40 v/v Retention time: 2.797 min Linearity range: 5-25 µg/ml Detection wavelength: 215nm
4.	Rapid HPLC analysis of the antiepileptic lamotrigine and its metabolites in human plasma [5]	Column: C8 RP column Flow rate:1.3 ml/min Mobile phase: methanol: phosphate buffer (pH 3.5) containing 0.17% triethylamine 24:76v/v Linearity range: 0.1-15.0 µg/ml Detection wavelength: 220 nm
5.	Development and validation of stability indicating RP-HPLC method for simultaneous estimation of lamotrigine and valproic acid and their dosage forms in biorelevant dissolution media [6]	Column:C8 cartridge precolumn (4 × 3 mm id, 5µm) Flow rate: 1 ml/min Mobile phase: phosphate buffer pH 3.5: acetonitrile (30:70) Linearity range: 20 to 100 µg/ ml and 120 to 600 µg/ml Detection wavelength: 220 nm Injection volume: 10µl
6.	Development and Validation of a Dissolution Method for Lamotrigine Tablets by UPLC [7]	Column:acquity UPLC BEH Shield RP18, 2.1 X 50mm. Flow rate: 0.3 ml/min Mobile phase: buffer: acetonitrile (75:25) %v/v Linearity range: 100 to 300 µl/ml Injection volume: 2.0 µl Detection wavelength: 310 nm Column temperature: 30 Dissolution test conditions: Apparatus: USP apparatus II (paddle) RPM: 75RPM Dissolution medium: 0.1 N HCl Media volume: 900 ml

		Time: 30 minutes **Sample collection volume: 10ml** **Temperature: 37.0 ±0.5^0 C**
7.	**Simple and sensitive spectrophotometric determination of lamotrigine in pure form and in dosage form [8]**	**Two methods are used based on the measurement of absorbance of lamotrigine either inmethod A or method B at 225 nm.** **Method A:** **Solvent: 0.1M H_2SO_4** **Wavelength: 225 nm** **Linearity range: 0.5 to 5.0µg/ml** **Method B:** **Solvent: methanol** **Wavelength: 225nm** **Linearity range: 1.25 to 12.5 µg/ml**
8.	**Lamotrigine analysis in blood and brain by high-performance liquid chromatography [9]**	
9.	**Bromometric analysis of Lamotrigine, Minoxidil$_2$, Cefixime [10]**	**Two spectrophotometric methods for determination of L, M and C in bulk and pharmaceutical dosage forms (1) insitu generated bromine as oxidizing agent, (2) methyl orange or methylene blue as chromogenic agents.** **Lamotrigine:** **Wavelength (methylene blue): 667nm** **Linearity range (methyl orange & methylene blue): 4.0-30.0, 5.0-40.0 µg/ml** **Minoxidil:** **Wavelength (methylene blue): 667nm** **Linearity range (methyl orange & methylene blue): 0.5-4.0, 0.5-7.0 µg/ml** **Cefixime:** **Wavelength (methylene blue): 747nm** **Linearity range (methyl orange & methylene blue): 0.-5.0, 2.0-5.5 µg/ml**
10.	**Optimization of a high-efficiency liquid chromatography technique for measuring lamotrigine in human plasma [11]**	**Column: Kromasil-100 C18, 15µm-15×0.4 cm and a LiChroCART-RP18e-3µm, 5.5×0.4 cm** **Flow rate: 1ml/min** **Mobile phase: 0.1M KH_2PO_4:triethylamine: methanol (62:3:35v/v)** **detected wavelength: 206nm**
11.	**First HPLC method for the simultaneous quantification of levetiracetam, zonisamide, lamotrigine, pentylenetetrazole and pilocarpine rat plasma and brain [12]**	**Column: LiChroCART purpospher star column (C18, 55mm × 4 mm; 3µm particle size)** **Mobile phase: acetonitrile (7.5%) and a mixture (92.5%) of water-triethylamine (99.5:0.05% v/v) at pH 6.4 adjusted with 85% ortho-phosphoric acid.** **Injection volume: 20µl** **Detected wavelength:** **Zonisamide:240nm** **LEV, PTZ, LTG and IS: 215nm** **Temperature: 30 ºC** **Flow rate: 1ml/min**
12.	**Development and validation of analytical method for simultaneous estimation of lamotrigine and clozapine in synthetic mixture by absorbance correction method [13]**	**Linearity range:** **Lamotrigine: 1-5µg/ml** **Clozapine: 6-30µg/ml** **Detected wavelength:** **Lamotrigine: 370nm** **Clozapine: 360nm**
13.	**Development and validation of a stability indicating HPLC assay method for determination of lamotrigine in tablet formulation [14]**	**Column: Phenomenex Luna C8 column (250mm × 4.6 mm *i.d.*, 5µm particle size)** **Mobile phase: methanol: 0.01M ammonium acetate pH4.3(50:50, v/v)** **Flow rate: 0.8ml/min** **Injection volume: 20µl** **Detected wavelength: 225nm** **Linearity range: 40-160 µg/ml**
14.	**Development of a dissolution test for lamotrigine in tablet form using an ultraviolet method [15]**	**Column: RP-18 octadecyl saline column (150 mm × 4.6 mm i.d., particle size 5 µm)** **Mobile phase: triethylamine solution 0.3% pH 4.0 (adjusted with 10% phosphoric acid): methanol (62:38 v/v)** **Flow rate: 1.0ml/min** **Injected volume: 20µl** **Column temperature: 25^0 C** **Detection wavelength: 267nm** **Linearity range: 10.0 to 80.0 µg/ml**

		Dissolution test conditions: **Paddle speed: 50rpm** **Dissolution medium: 0.01M hydrochloric acid**
15.	**HPLC method for simultaneous determination of five antileptic drugs in rat plasma [16]**	**Five antileptic drugs used in rat plasma (lamotrigine (LTG), oxcarbazepine (OXC), carbamazepine (CBZ), its major metabolite carbamazepine 10,11-epoxide (CBZE) and phenytoin (PHT)** **Column: Nova-Pak® C18 analytical column(250 × 4.6 mm, 4 µm)** **Column temperature: 30 ^{0}C** **Mobile phase: potassium dihydrogen phosphate buffer: acetonitrile: 2-propanol (63:22:15 v/v/v)** **Flow rate: 1ml/min** **Detected wavelength: 220nm**
16.	**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 [17]**	**Column: reverse phase C18** **Mobile phase: water: methanol: acetonitrile: triethylamine (68.7:25:6:0.3, v/v/v/v; pH 6.5)** **Detected wavelength: 237nm** **Linearity ranges:** **Phenobarbital: 0.25–100 g/mL** **Primidone: 0.4–50 g/mL** **Phenytoin: 0.5–50 g/mL** **Carbamazepine and lamotrigine: 0.1–50 g/mL** **Oxcarbazepine: 0.1–25 g/mL** **Regression coefficient: greater than 0.992**
17.	**Simultaneous determination of lamotrigine, phenobarbitone, carbamazepine and phenytoin in human serum by high-performance liquid chromatography [18]**	**Column: NOVA PAK C18 column (250mm × 4.6mm, 5µm Hypersil ODS)** **Mobile phase: 10mM phosphate buffer: methanol: acetonitrile: acetone (55:22:12:11)** **Detected wavelength: 210nm** **Flow rate: 1.2ml/min**
18.	**LC and UV methods for Lamotrigine determination in pharmaceutical formulation [19]**	**Column: ACE RP-18 octadecyl silane column (150 mm × 4.6 mm i.d., particle size 5 µm)** **Mobile phase: 0.3% trimethylamine with pH 4.0: methanol (62:38V/V)** **Detection wave length: 279nm** **Flow rate: 1 ml/min** **Injection volume: 20µl** **Run time: 10min**
19.	**Development and validation of the analytical method by high performance liquid chromatography (HPLC) for Lamotrigine raw material [20]**	**Column: Inertsil ODS-3, diameter of 4.6 mm and length of 250mm** **For intermediate precision and robustness another C18 column Agilent Zorbax Eclipse XDB** **Mobile phase: potassium monobasic buffer 0.05mol/lit (pH 6.8): acetonitrile (75:25v/v)** **Temperature: 25^0 C** **Injection volume: 5µl** **Detected wavelength: 210nm**
20.	**Development and validation of a stability indicating RP-HPLC assay method for determination of lamotrigine in tablet formulation [21]**	**Column:Thermo C18 column (250mm × 4.6mm i.d., 5µm particle size)** **Mobile phase: acetonitrile: buffer (40:60)** **1.75 gm KH_2PO_4 in 1000 ml of water and 1ml of TEA and adjust the pH 6 with OPA** **Flow rate: 1.0ml/min** **Detection wavelength: 225nm** **Linearity range: 5-25 µg/ml** **Correlation coefficient: 0.999**
21.	**Simultaneous liquid chromatographic determination of lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in plasma of patients with epilepsy [22]**	**Column: Synergi 4µm Hydro-RP, 150 mm × 4 mm internal diameter** **Mobile phase: potassium dihydrogen phosphate buffer (50mM pH 4.5): acetonitrile/methanol 3/1(65:35,v/v)** **Detected wavelength: 210nm** **Linearity range:** **Lamotrigine: 1-20 µg/ml** **Monohydroxy carbamazepine: 2-40 µg/ml** **Felbamate: 10-120 µg/ml** **Flow rate: 1.0 ml/min**
22.	**Four spectrophotometric methods for simultaneous determination of carbazepine and lamotrigine in**	***Method I*: solving the two simultaneous equation (SEQ) based on total absorbance according to beer's law**

	binary mixtures and urine samples [23]	***Method II:*** **dual wavelength method (DWSP) Absorbance difference between 304 and 313 was measurable for carbazepine, but was zero for lamotrigine. Likewise, the absorbance between 282 and 290 nm was significant for lamotrigine, but zero for carbazepine.** ***Method III:*** **zero-crossing first derivative method (ZCDSP) using the amplitudes at 308.9 and 286.6 nm for carbazepine and lamotrigine** ***Method IV:*** **ratio derivative spectroscopy (RDSP)** **Correlation coefficient: range between 0.9990- 0.9997** **Detection limit was mostly less than 0.4 µg/ml in the case of ZCDSP AND RDSP were between 0.01-0.2 µg/ml**
23.	**A validated reversed-phase high-performance liquid chromatography method for simultaneous determination of five antiepileptic drugs used in the treatment of lennox-gastaut syndrome in their pharmaceutical dosage forms [24]**	**Column: RESTEK C18 column (5µm, 250nm × 4.6 mm)** **Mobile phase: acetonitrile: water (5545 v/v)** **Flow rate: 1 ml/min** **Detection wavelength: 210nm** **Linearity range:** **Rufinamide and diazepam: 2-40 µg/ml** **Lamotrigine and clozepam: 0.5-40 µg/ml** **Valproic acid: 36-180 µg/ml**
24.	**Determination of lamotrigine in human plasma using liquid chromatography-tandem mass spectrometry [25]**	**Column: Cadenza CD-C18 column (100 × 2mm, 3µm)** **Mobile phase: 0.1% formic acid in water and acetonitrile (2/1, v/v)** **Temperature: 40^0 C** **Flow rate: 0.2ml/min** **Injection volume: 20µl** **Retention times of lamotrigine and internal standard: 1.6 and 2.0 min** **Run time: 3.5 min** **Mass spectrometry optimal parameters** ***Nebulizer gas (nitrogen):*** **3.0 L/min** ***DL temperature*****: 250^0 C** ***Heat block temperature:*** **400^0 C** ***Drying gas (nitrogen):*** **15.0 L/min**
25.	**RP-HPLC Method Development and Validation of Lamotrigine [26]**	**Column: Supelco C18 (25cm × 4.6mm and i.d., 5µm)** **Mobile phase: methanol: potassium dihydrogen orthophosphate (65:35 v/v)** **Flow rate: 1 ml/min** **Detected wavelength: 270nm** **Retention time: 3.7 min** **Linearity range: 20-100 µg/ml**
26.	**Development and validation of a new HPLC method for determination of lamotrigine and related compounds in tablet formulations [27]**	**Column: C18 µ- Bondapack column (250mm × 4.6 mm)** **Mobile phase: acetonitrile: monobasic potassium phosphate solution (35:65 v/v)** **Flow rate: 1.5 ml/min** **Column temperature: 40^0 C** **Detected wavelength: 210nm**

CONCLUSION

For the analysis of Lamotrigine or its combination in pharmaceutical formulations different analytical techniques such as UV, HPLC, UPLC, Bromometric analysis, liquid chromatography-tandem mass spectrometry. Mostly HPLC and UV-Spectrophotometry was used and mobile phase are acetonitrile, methanol, potassium dihydrogen phosphate, phosphate buffer and their flow rate are 1.0ml/min. retention times is less than 5 min. for the dissolution method they used the 0.01N hydrochloric acid.

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