Review Article

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A Review on Methods of Analysis of Eslicarbazepine Acetate

Asif Husain[1], Ahmad Nawed[2], Sahil Haque[3], Abdul Qadir[4], Aqsa Khan[5], Md Khurshed Alam[6]

[1]Professor, [2-6]UG Scholar, Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110 062, India.

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Abstract

Antiepileptic drugs are used to treat neurological disorders characterized by recurrent epileptic seizures. Eslicarbazepine acetate (ESA), a promising antiepileptic agent, acts by blocking the voltage-gated sodium channels. It is a prodrug and converted to eslicarbazepine. ESA is available in the market under different trade names like Aptiom, Zebinix, and Exalief. This review sheds light on the properties and essential ESA analysis methods like IR, HPLC, LC-MS, HPTLC, and UPLC.

Keywords: Epilepsy; ESA, Assay; UV; HPLC; MS.

Introduction

Epilepsy is a neurological disorder that is characterized by a periodic and unpredictable expression of seizures. Epileptic seizures are produced due to anomalous extreme or synchronous neuronal action in the brain, and episodes of seizures may vary from short to long periods. Nervous system infection, prenatal factors, headtrauma, delay development, and genetic issues are factors behind the etiology of seizures.[1-3]

Antiepileptic drugs are used to treat epileptic conditions. Nevertheless, despite the existing treatment, 30% of adult patients have uncontrolled seizures.[4] To reduce the side-effects and drug resistance there is an urgent need to develop newer, safer and effective drug(s) to treat epilepsy. Various combination therapies are used now to create the synergistic effect and reduce the overall side effects of existing drugs.[5,6] Patients suffering from the epileptic condition have a poor quality of life, which will further lead to disability and morbidity.[7]

Various signs and symptoms[8] associated with epilepsy include temporary confusion, staring spells, uncontrolled jerking, psychic symptoms, and loss of consciousness, etc. (Fig 1).

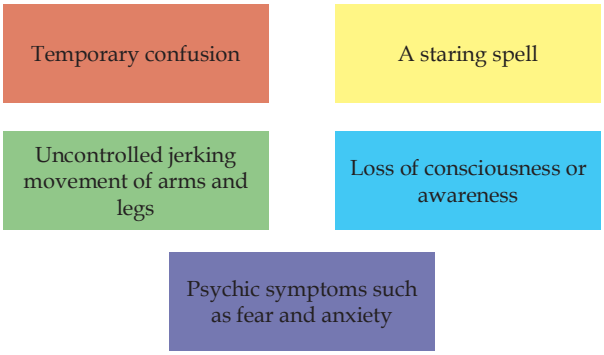

Fig. 1: Some sign and symptoms of epilepsy.

There are basically two types of seizures (Fig 2), one is focal seizure, also known as partial or localized seizure in which only one hemisphere of brain gets affected initially. Another one is generalized seizure, which occurs when there is abnormal electrical activity in both halves of the brain simultaneously.[9]

Corresponding Author: Asif Husain, Professor, Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110 062, India.
Email: drasifhusain@yahoo.com

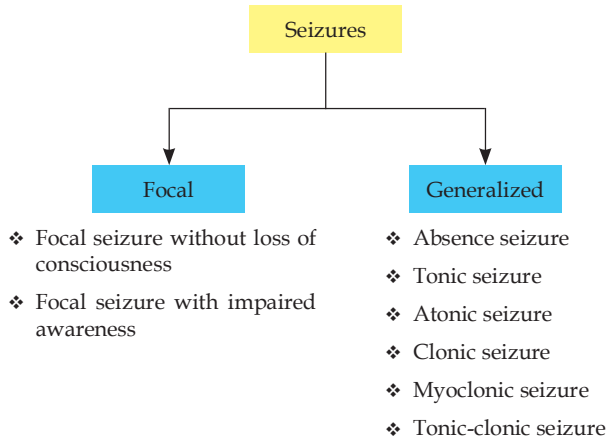

Fig. 2: Types of seizure.

Eslicarbazepine acetate (ESA) is one of the most prominent anticonvulsant agents. It is a new broad spectrum antiepileptic drug, and effective against partial and generalized tonic-clonic seizures as a single drug or in combination with another antiepileptic drug. ESA acts by blockage of the voltage-gated sodium channel. It has half-life of 20-24 h. It is a once-daily antiepileptic drug developed by European Medicines Agency (ZebinixTM).[10]

ESA is available under the trade name Aptiom, Zebinix, and Exalief. ESA is a prodrug that is converted to eslicarbazepine (S-licarbazepine: an active metabolite of oxcarbazepine). ESA is chemically known as (S)-10-acetoxy-10, 11-dihydro-5H-dibenz [b,f] azepine-5-carboxamide.The structures of Eslicarbazepine and its prodrug ESA are presented in Fig 3.

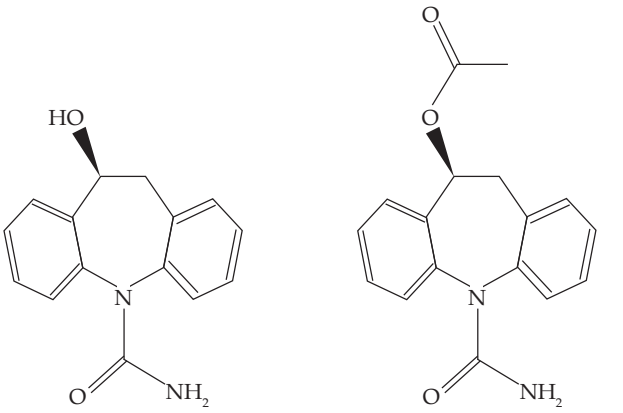

Fig. 3: Structure of Eslicarbazepine and ESA.

ESA is absorbed to an extent upto 90% upon oral administration. It has improved tolerability profile.[11] The physical properties of ESA[12] are presented in Table 1.

Table 1: Physical properties of ESA.

Molecular formula	$C_{17}H_{16}N_2O_3$
Molecular weight	296.32
Melting point	184-187°C
Appearance	White to off white crytalline powder
Nature	Non-hygroscopic
Odour	Odourless
Solubility	Freely soluble in dichloromethane, sparingly soluble in acetone, acetonitrile and methanol, slightly soluble in absolute ethanol.

Assay Methods

Analytical method development has a great role in the discovery, development and manufacture of pharmaceuticals.Various methods for the analysis of eslicarbazepine in different pharmaceutical formulations are reported here:

1. Reverse phase High- performance liquid chromatography (RP-HPLC)
2. High performance liquid chromatography-Ultraviolet spectrophotometry (HPLC-UV)
3. Reverse phase-high performance liquid chromatography ultraviolet spectrophotometry (RP-HPLC-UV)
4. Liquid chromatography tandem mass spectrometry (LC-MS/MS).
5. High-performance thin layer chromatography (HPTLC)
6. Ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS).

Reverse phase High- performance liquid chromatography (RP-HPLC)

HPLC is an extremely versatile technique of analysis in which analytes are separated by passing through a column packed with micrometer-sized particles. Reverse phase chromatography is used now a days due to its simple, versatile, robust nature.It can handle compound of varied polarity and molecular mass.[13,14]

Compounds such as proteins, peptides and nucleic acid can be easily separated and recovered by RP-HPLC.[15] The separation takes place by hydrophobic binding interaction between the solute molecule in mobile phase and the immobilised

hydrophobic ligand that is stationary phase.[16,17]

In 2012 Patel D. et al. developed a simple, robust, rapid, and precise method for quantitative measurement of eslicarbazepine acetate in tablets by RP-HPLC method. Inertsil ODS-2 (15x4.6mm), 5µm is the column used in this method. Acetonitrile: methanol in the ratio 60:40v/v was used as solvent mixture with a flow rate of 1.0ml/min and detector wavelength of 230nm. The assay showed 99.17% accuracy. This method can be used for routine quality control test and it showed good validation parameters. A linear relationship between concentration and result was obtained.[18]

Srinivas M. et al. in 2012 reported a new stability-indicating gradient RP-HPLC method to measure the content of ESA in bulk drug and pharmaceutical dosage forms. Zorbax SB C-18 (150mmx 4.6mm, 3.5µm) column was used with mobile phase (gradient mixture of solvent A and B). Solvent A: mixture of 0.01M KH_2PO_4 and methanol in ration 80:20 and solvent B: acetonitrile: methanol: water ratio 75:5:20v/v. The drug when subjected to stress conditions degraded significantly in acid, base, neutral hydrolysis and oxidative conditions. The degradation product was separated from impurities. This method was found to be suitable for determination of ESA in drug product.[19]

Singh M. et al. in 2013 estimated ESA in bulk drug and tablet dosage form by developing a simple, convenient, accurate, precise and reproducible RP-HPLC method.Dionex C18 column (250×4.6 mm, 5 µm particle size) was chosen for chromatographic separation with mobile phase methanol and ammonium acetate (0.005 M) in the ratio of 70:30 v/v.The limit of detection and quantification were found to be 3.144 and 9.52 µg/ml, respectively.The amount of ESA in bulk and tablet dosage form was found to be 99.19 and 97.88%, respectively.[20]

Kallam S. R. et al. in 2015 designed and validated a simple, rapid, selective, precise and accurate isocratic RP-HPLC method for estimation of ESA in bulk and pharmaceutical dosage form using Inertsil ODS 3V 150 mm, 4.6 mm, 5µm chromatographic column with mobile phase methanol and acetonitrile in the ratio of 500:250 with a flow rate of 1.5 mL/min. ESA was exposed to thermal, photolytic, hydrolytic, basic and oxidative stress conditions. When exposed to basic conditions, it degraded completely.The photodiode array (PDA) detection methodwas used to get peak homogeneity data of ESA.[21]

High performance liquid chromatography-Ultraviolet spectrophotometry (HPLC-UV)

Here high-performance liquid chromatography coupled with ultra violet detection (HPLC-UV) is used to quantify ESA.Gilberto Alves et al. in 2007 developed a simple and reliable chiral reversed phase HPLC-UV method to determine ESA in human plasma. By the use of solid phase extraction, the analytes and internal standard were extracted from plasma. Chromatographic separation was done by isocratic elution with water-methanol (88:12v/v) solvent system at 0.7mL/min flow rate. The column used was Lichro CART 250-4ChiraDex (5µm). Compounds were detected at wavelength of 225nm. Promising results were obtained and the method was found to be useful for clinical research and for therapeutic drug monitoring odESA and its metabolites.[22]

In 2010 Fortuna A. et al. created and validated a simple, selective and accurate HPLC-UV method to determine antiepileptic drugs which are structurally related: carbamazepine, oxcarbazepine, eslicarbazepine and their metabolites in human plasma. The solid-phase extraction and reverse phase C18 column was used in this method. Water: methanol: acetonitrile (64:30:6) ratio was used as mobile phase and pumped at the rate of 1mL/min. The method showed accurate, precise, selective and linear relation over concentration range of 0.05-30µg/mL. This method can also be used for therapeutic drug monitoring.[23]

Reverse phase-high performance liquid chromatography ultraviolet spectrophotometry (RP-HPLC-UV)

This technique (RP-HPLC-UV) is a good method for quantification of ESA and detection of impurities present with ESA. Thomas S.et al in 2012 developed a novel, sensitive, selective and stability indicating method for the determination of potential impurities of ESA. The method used high performance liquid chromatography liquid chromatography coupled with electrospray ionization, ion trap mass spectrometry (LC/ESI-IT/MS/MS). The developed method was validated as asper ICH guidelines. This method was claimed to be highly efficient, selective, sensitive and stable.[24]

Liquid chromatography tandem mass spectrometry(LC-MS/MS)

It is an important couple technique which combines the capabilities of physical separation by liquid chromatography and mass analysis by mass spectrometry.Liquid chromatography separates the mixture with multiple components.By the use of mass spectrometry one can predict structural identity of individual component of a mixture. This technique is successfully applied in different fields like- biotechnology, food processing, agrochemical, cosmetics, environmental monitoring and pharmaceutical.[25,26]

Li T. et al in 2019 created and validated a specific, sensitive and straightforward method to measure the content of ESA in human plasma. Hanbon ODS-2 C18 column (150mm × 2.1mm, 10µm) was used for chromatographic separation with isocratic elution using 10mM ammonium acetate. The mobile phase was formic acid and acetonitrile (72:28v/v).[27]

Loureiro A. I. et al. in 2011 developed a sensitive and specific enantioselective LC-MS/MS method to quantify ESA, eslicarbazepine, oxcarbazepine R-licarbazepine in human plasma. Daicel CHIRALCEL OD-H column (5µm, 50mm × 4.6mm) with mobile phase n-hexane:ethanol/isopropyl alcohol (66.7:33.3 v/v)with flow rate 0.8 ml/min was used for the analysis. The compoundswere quantified by positive ion electrospray ionization mass spectrometry. The accuracy was found in between 98.7% to 107.2% for all compounds.[28]

Kroner G. M. et al. in 2020 developed a LC-MS/MS method to analyse metabolite of oxcarbazepine and eslicarbazepine, licarbazepine by the use of 13C-labelled form of compound as internal standard. To compare the results between adult and paediatric patients, retrospective data analysis was used. This data showed similar distribution of oxcarbazepine/eslicarbazepine metabolite concentration in serum.[29]

High-performance thin-layer chromatography (HPTLC)

A number of enhancements have been made to TLC so that more accurate quantitative measurements can be performed with increased the resolution.[30]

Mohamed F. A. et al. in 2019 developed a simple and sensitive HPTLC method to analyse two binary mixtures of antiepileptic drugs. Mixture 1 containedESA and oxcarbazepine, while mixture 2 hadcarbazepine and oxcarbazepine. By the use of pre-coated silica gel HPTLC plates G60F24, both the mixtures were separated. Mobile phase used in this process was n-hexane: methylene chloride: ethanol: glacial acetic acid (50:40:10:0.1, v/v/v/v). At wavelength 217nm mixture1 was detected, and at 265nm mixture 2 was detected. The results showed good recovery rates from pharmaceutical tablet in the range 97.75 to 100.40%.[31]

Ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS)

This is a powerful quantification technique that combines the physical separation tendency of liquid chromatography with mass analysis tendency of mass spectrometry. The technique is highly sensitive, accurate, reliable and selective. It is efficiently used to identify and quantify the chemicals in a complex mixture.[32]

Iram F. et al. in 2018 developed an Ultra Performance Liquid Chromatography (UPLC) method for quantification of ESA and its metabolites. The developed method was found to be more acceptable as compared to conventional HPLC methods in term of rapid chromatographic separation, high analysis, excellent resolution and good sensitivity. All validation parameters gave acceptable results in terms of specificity, selectivity, linearity, precision, accuracy and robustness.[33]

Farouk F. et al. in 2017 developed a UPLC-MS/MS method for determining four compounds related to eslicarbazepine in plasma/serum. Intersil RP-HPLC column (250×4.6mm, 5µm) was used foe chromatographic separation with a mobile phase acetonitrile, methanol and 100mM ammonium acetate in water (32:3:65, v/v/v). Detection was performed using tandem massspectrometry.[34]

Husain A. et al. in 2020 developed a method of UPLC-MS/MS to analyze ESA and its metabolites such as cis-10,11-dihydro-5H-dibenz [b,f] azepine-10,11-diol in rat plasma. Identification of metabolite has become an important part of drug discovery process. The assay utilized precipitation technique using acetonitrile and 0.01M potassium dihydrogen phosphate (60:40 v/v) at a flow rate 0.2ml/min. The results showedthe efficiency of this method to identify and quantify the compounds.[35]

Fourier transform infrared spectroscopy (FT-IR Spectroscopy)

Prasad R. et al. reported a simple, precise and rapid FT-IR spectroscopic method for measurement of ESA in bulk and tablet formulations.Absorbance was done at 1726 cm-1 and evaluation of the drug component carried out using standard calibration plot. This method was proposed as cost-effective

method to estimate ESA in pharmaceuticals.[36]

Conclusion

Eslicarbazepine acetate (ESA) is an important antiepileptic drug. Analysis of ESA in different formulations is an important aspect of quality control. There are various methods available for the analysis of ESA. The majority of the reported modern methods are reproducible, rapid, cost-efficient and give accurate results. However, further research is required to find novel, accurate, reliable, rapid, cheap and easy to implement method(s) to determine ESA and its metabolites.

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