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REVIEW ARTICLE
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"ANALYTICAL METHODS FOR THE ESTIMATION OF SAFINAMIDE AND LEVODOPA: A COMPREHENSIVE REVIEW"

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ABSTARCT:
Parkinson's disease is a chronic neurological disorder that primarily impacts movement. It occurs when specific brain cells that produce a chemical known as dopamine begin to die. This results in common symptoms such as difficulties with balance, stiff muscles, lack of movement, and shaking. Parkinson's disease patients may also experience non-motor symptoms such as anxiety or depression, issues with memory, or trouble falling asleep. While there is no cure, various treatments have been developed to help manage the symptoms of Parkinson's disease. Management of PD is a growing field and targets new treatment methods, as well as improvements to old ones. Pharmacological, surgical, and therapeutic treatments have allowed physicians to treat not only the main motor symptoms of Parkinson's disease. Medications like levodopa and safinamide are widely used to manage its symptoms. Levodopa is the most effective treatment for improving motor function, as it helps replenish dopamine levels. Safinamide is an add-on therapy used in combination with levodopa, which works by inhibiting monoamine oxidase-B (MAO-B) and modulating glutamate release. This combination helps reduce motor fluctuations and "off" periods, improving patients' overall mobility and quality of life. Together, these treatments play a vital role in improving symptom control in individuals living with Parkinson's disease, though ongoing research continues to seek a true cure.

KEYWORDS: Parkinson's disease, Safinamide, Levodopa, Drug profile, Pharmacopeial methods, Validation

1. INTRODUCTION

Parkinson's disease is a progressive neurological disease first described in 1817 by James Parkinson that mostly restricts a person's movement.[1] After Alzheimer's disease (AD), Parkinson's disease (PD) is the second most common neurodegenerative diseases.[2] It happens when nerve cells in a part of the brain called the substantia nigra stop working properly and die. These cells produce a chemical called dopamine, which helps control movement. It may be due to a mix of genetic factors (inherited from family) and environmental exposures (like certain toxins). Right now, there is no cure, but treatments

like medications, physical therapy, and sometimes surgery can help manage the symptoms and improve quality of life.[3]

Common medications used to treat Parkinson's disease include Levodopa, Carbidopa, Amantadine, Bromocriptine, safinamide, Benserazide etc …

Levodopa, a treatment for Parkinson's disease, was approved by the U.S. Food and Drug Administration (FDA) in 1970. The modern synthetic form of levodopa was first developed and tested in the West in the 1960s. The first levodopa combination drug, carbidopa/levodopa, became commercially available globally in 1975. Research on Parkinson's disease in India began to be published in indexed international journals from 1988 onwards, with a growing number of publications and studies from the late 20th century.[5]

Levodopa, the precursor of dopamine, was first developed for the treatment of PD in the 1960s and continues to be the most-effective therapeutic agent for PD in 2020.[6]

It helps manage the motor symptoms caused by low levels of dopamine, a chemical that controls movement. It works in two ways:

- Replaces dopamine – Levodopa is converted into dopamine in the brain. Since Parkinson's disease causes dopamine loss, levodopa helps restore dopamine levels and improving movement control.
- Improves motor symptoms –

such as:

Tremors (shaking)

Bradykinesia (slowness of movement)

Muscle stiffness

Freezing episodes and walking difficulties[7]

To overcome these challenges, The European Commission and the US Food and Drug Administration (FDA) have approved safinamide (Xadago®), a novel drug with both dopaminergic and non-dopaminergic effects, as an adjuvant treatment for patients with mid- to late-stage Parkinson's disease (PD).[8]

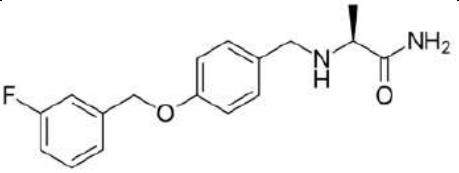
Safinamide is a pill used in Parkinson's disease to help with motor fluctuations (shaking hands or fingers, slow walking, delayed body movements). It works in two ways:

- Inhibits MAO-B enzyme – This increases dopamine levels in the brain, improving movement symptoms.
- Reduces glutamate release – At higher doses (100 mg), it blocks sodium and calcium channels, which decreases glutamate activity. This helps reduce dyskinesia (uncontrolled movements) and may improve non-motor symptoms like mood, thinking, and pain.[9]

Given their pharmacological importance, the precise assessment of levodopa and safinamide is a critical component of pharmaceutical research, quality assurance, and therapeutic monitoring. This review discusses the role of accurate quantification in drug development, highlights analytical challenges, and outlines validated methodologies used for these two key APIs.

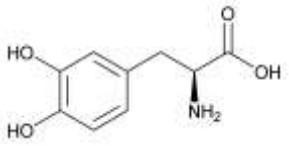
2. DRUG PROFILE

2.1. Safinamide[10,11]

IUPAC Name	(S)-(+)-2-[4-(3-Fluorobenzyl)oxybenzyl]aminopropanamide
Molecular Formula	C17H19FN2O2
Chemical Structure	
Molecular Mass	302.34 g/mol
Description	white to off-white crystalline powder
Solubility	slightly soluble in water, but freely soluble in organic solvents such as methanol, ethanol, acetone, and dimethyl sulfoxide

pH and pKa Value	pH of 1% Solution: 5.0–6.5 9.2
Melting Point	129°C to 131°C
CAS number	133865-89-1
Mechanism of Action	Safinamide is a unique molecule with multiple mechanisms of action and a very high therapeutic index. It combines potent, selective, and reversible inhibition of MAO-B with blockade of voltage-dependent Na+ and Ca2+ channels and inhibition of glutamate release.

2.2. Levodopa[12,13]

IUPAC Name	(2S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid
Molecular Formula	C9H11NO4
Chemical Structure	
Molecular Mass	197.19 g/mol
Description	white crystalline powder
Solubility	Slightly soluble in water; practically insoluble in chloroform, in ethanol (95 per cent) and in ether. Freely soluble in 1M hydrochloric acid but sparingly soluble in 0.1M hydrochloric acid.
pH and pKa Value	pH-5.5 pKa_1 (carboxyl group –COOH): 2.29 pKa_2 (phenolic –OH): 8.72 pKa_3 (amino group $–NH_3^+$): 9.74
Melting Point	276°C to 295°C
CAS number	59-92-7
Mechanism of Action	Degeneration of the substantia nigra occurs in patients with Parkinson disease. This condition results in the disruption of the nigrostriatal pathway and thus decreases the striatal dopamine levels. Unlike dopamine, levodopa can cross the blood-brain barrier (BBB). Levodopa converts to dopamine in both the CNS and periphery.

3. LITERATURE SURVEY

3.1 SAFINAMIDE

3.1.1 Reported Methods of Safinamide (Alone):

Title	***Name of Journal with year of Publication***	**Summary**	**Ref. No.**
A validated chiral liquid chromatographic method for the enantiomeric separation of safinamide mesylate, a new anti-Parkinson drug	*Journal of Pharmaceutical and Biomedical Analysis*, **2011**	**RP-HPLC** **Column:** Chiralcel OD-RH column (150 mm × 4.6 mm, 5 µm), cellulose-based **Mobile Phase:** 300 mM sodium dihydrogen phosphate buffer (pH 3.0): methanol: acetonitrile — 65: 25: 10 (v/v/v) **Detection wavelength:** UV (likely ~254 nm or UV-PDA) **Flow Rate:** 1 mL/min	14
Determination of Genotoxic Impurities in Safinamide Mesylate by LC/MS	*Metrology Science and Technology*, **2022**	**LC–MS** **Column:** YMC-Triart C18 (100 mm × 4.6 mm, 3 µm) **Mobile Phase:** Gradient of 0.1 % formic acid in water and in methanol **Detection wavelength:** LC–MS (positive-ion MRM) **Flow rate:** 0.4 mL/min	15
Development and Validation of Stability Indicating RP-HPLC Method for Determination of Safinamide Mesylate	*Jordan Journal of Pharmaceutical Sciences,* **2020**	**RP-HPLC** **Column:** Hypersil BDS C18 (250 × 4.6 mm, 5 µm) **Mobile Phase:** Methanol: Phosphate buffer (pH 6.8) – 80: 20 (v/v) **Detection wavelength:** UV at 226 nm **Flow Rate:** 1.0 mL/min	16

		Retention time: 2.305 min	

3.1.2 Reported Methods of Safinamide in combination with other drugs:

Determination of RP-HPLC Method for Safinamide: Its Bulk and Tablet Dosage Form	*A Journal of Drug Formulation, Development and Production,* **2018**	**RP-HPLC** **Column:** Agilent C18 (150 × 4.6 mm, 5 µm) **Mobile Phase:** Methanol: Acetonitrile – 60: 40 (v/v), pH 3 adjusted with orthophosphoric acid **Detection wavelength:** UV at 235 nm **Flow Rate:** 1.0 mL/min **Retention Time:** ~2.305 min	17
Development And Validation Of RP-HPLC Method For Determination of Safinamide Mesylate and Nasal Spray Formulation	*African Journal of Biomedical Research,* **2024**	**RP-HPLC** **Column:** Hypersil BDS C18 (250 × 4.6 mm, 5 µm) **Mobile Phase:** Methanol: Phosphate buffer (pH 6.8) – 80: 20 (v/v) **Detection wavelength:** UV at 226 nm **Flow Rate:** 1.0 mL/min **Retention time:** 4.68 min	18
RP-HPLC Method Development and Validation for the Estimation of Safinamide in API form and marketed formulation	*International Journal of Research,* **2021**	**RP-HPLC** **Column:** ODS RP C18column (15mm x 4.6mm) 5µm **M.P:** Methanol: Acetonitrile (80:20v/v) **Detection wavelength:**282nm **Flow rate**:1.0 ml/min **Retention Time:** 2.545 ± 0.3 min	19
RP-HPLC Method Development and Validation for Determination of Safinamide in Bulk and Pharmaceutical Formulation	*International Journal of Pharmaceutical Sciences,* **2025**	**RP-HPLC** **Column:** C18column (100mmX 4.6mm) 2.5µm **M.P:** ACN and Water (0.1% OPA) **Detection wavelength:** 225 nm **flow rate:** 1.0 mL/min **Retention Time:** 4.444 Min	20

3.2 LEVODOPA

3.2.1 Official Methods of Levodopa (Alone):

Sr.no	Pharmacopeia	Method Description	Ref no.
1	United States Pharmacopeia (USP), **2024**	**Assay by Liquid Chromatography** **Mobile phase:** Tetrahydrofuran and Diluent (3:97) **Column:** 4.6-mm × 25-cm; 5-µm L1 packing **Detection wavelength:** UV 280 nm **Flow rate:** 1 mL/min **Injection volume:** 20 µL	21

3.2.2 Official Methods of Levodopa in combination with other drugs:

Sr.no	Pharmacopeia	Method Description	Ref no.
1	United States Pharmacopeia (USP), **2024**	**Assay by Liquid Chromatography** **Mobile phase:** 11.0 g/L of monobasic sodium phosphate in solution, prepared as follows. Transfer a sufficient quantity of monobasic sodium phosphate into a container, and dissolve in water, using 95% of the total volume. Add 0.13% of the total volume of *Diluent*, and adjust with phosphoric acid to a pH of 2.8. Transfer to a suitable volumetric flask, and dilute with water to volume. **Column:** 3.9-mm × 30-cm; 10-µm packing L1 **Detection wavelength:** UV 280 nm **Flow rate:** 2 mL/min **Injection volume:** 20 µL	22
2	United States Pharmacopeia (USP), **2022**	**Assay by Liquid Chromatography** **Mobile phase:** Dissolve 11.0 g of monobasic sodium phosphate monohydrate in 1 L of water. Add 1.3 mL of *Solution A(0.24 g/L of*	23

		sodium 1 decanesulfonate in water), and adjust with phosphoric acid to a pH of 2.8. **Column:** 4.6-mm × 15.0-cm; 5-µm packing L1 **Detection wavelength:** UV 280 nm **Flow rate:** 2 mL/min **Injection volume:** 20 µL	
3	Indian Pharmacopoeia (IP), **2018**	**Assay by liquid chromatography** **Mobile phase:** a mixture of 0.13 volumes of the final volume of a buffer solution prepared by dissolving 0.24 g *of sodium 1-decanesulfbnate* in 1000 ml of *water* and 95 volumes of the final volume of a buffer solution prepared by dissolving 11.6 g of *monobasic sodium phosphate* in 1000 ml of *water,* adjusted to pH 2.8 with *orthophosphoric acid.* Dilute with *water* to final volume **Column:** 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 gm) **Detection wavelength:** UV 280 nm **flow rate:** 2 mL/min **injection volume:** 20 µL	24

3.2.3 Reported Methods of levodopa (Alone):

A new validated HPLC method for the determination of levodopa: Application to study the impact of ketogenic diet on the pharmacokinetics of levodopa in Parkinson's participants	*Wiley analytical science*, **2019**	**HPLC** **Column:** Zorbax Eclipse XDB-C18 (standard analytical column). **Mobile Phase:** 20 mM KH_2PO_4 buffer (pH 2.5): Methanol = 95: 5 (v/v) **Mode:** isocratic **Detection Wavelength:** UV at 230 nm. **Flow Rate:** 1.0 mL/min.	25

3.2.4 Reported Methods of Levodopa in combination with other drugs:

Analytical method for simultaneous quantification of levodopa and carbidopa in the injectable oleogel formulation by HPLC	*BMC Chemistry Part of Springer Nature,* **2025**	**simultaneous LD + CD** **Column:** Luna-C18 (250 × 4.6 mm, 5 µm). **Mobile Phase:** **Phase A:** 30 mM potassium phosphate + acetonitrile (95:5, v/v) with 35 mM tetrabutylammonium hydrogen sulphate (ion-pairing agent) **Phase B:** 30 mM potassium phosphate + acetonitrile (50:50, v/v) **Detection Wavelength:** UV at 280 nm. **Flow Rate:** 1.0 mL/min **Retention Times:** Levodopa ~3.05 min; Carbidopa ~3.64 min.	26
RP-HPLC Method development, Validation and Forced Degradation for Simultaneous estimation of Benserazide HCl and Levodopa in a Marketed Formulation	*International Journal of PharmTech Research,***2020**	**RP-HPLC** **Column:** Cosmosil C18 (250 × 4.6 mm). **Mobile Phase:** Phosphate buffer (pH 2): acetonitrile = 97: 3 (v/v). **Detection Wavelength:** UV at 210 nm **Flow Rate:** 1.0 mL/min **Retention Times:** Benserazide ~3.1 min; Levodopa ~6.6 min.	27

CONCLUSION

The estimation of safinamide and levodopa is critical for ensuring the quality, safety, and therapeutic efficacy of pharmaceutical formulations and for guiding effective clinical use in Parkinson's disease management. A broad spectrum of analytical methodologies—ranging from traditional spectrophotometric techniques to advanced chromatographic and hyphenated methods such as HPLC, RP-HPLC, and LC–MS/MS—has been employed for their individual and combined determination. Each method carries distinct advantages and limitations in terms of sensitivity, selectivity, cost, and

operational complexity. While both safinamide and levodopa have well-established individual estimation methods, simultaneous estimation remains limited, largely due to their contrasting physicochemical properties—safinamide being moderately lipophilic and levodopa highly polar and oxidation-prone. This highlights a pressing need for further research and method optimization to enable robust, accurate, and reproducible simultaneous analysis. Among available approaches, reversed-phase HPLC and LC–MS/MS stand out as the most promising tools, offering superior sensitivity, selectivity, and precision suitable for pharmacokinetic profiling, bioequivalence studies, and quality control testing. Crucially, rigorous method validation following international regulatory guidelines (ICH, FDA, EMA) ensures reliability and reproducibility of results, supporting both pharmaceutical development and clinical monitoring.

In conclusion, ongoing innovation and refinement of analytical methodologies for safinamide and levodopa will continue to play a pivotal role in advancing pharmaceutical research, ensuring therapeutic consistency, and ultimately improving patient outcomes in Parkinson's disease.

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