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Mini review

Current analytical approaches for safinamide determination in pharmaceuticals and biological samples: a brief review

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ABSTRACT

Neurodegenerative disease, which mostly affects the elderly, is now one of the leading causes of death and sickness worldwide. Safinamide mesylate (SAF) is a medication that inhibits the enzyme monoamine oxidase-B (MAO-B). It is used with levodopa/carbidopa to enhance the treatment of Parkinson's disease. As SAF was recently approved in many countries such as the US (2017), Canada, and India (2019), the published analytical methods are still limited. All the reported spectroscopic or electrochemical methods devoted to pharmaceuticals or spiked plasma didn`t extend to real samples. Meanwhile, hyphenated techniques, such as chromatographic ones, were extended to the analysis of real clinical samples and used in stability-indicating assay studies. In this context, this review compiles the various analytical techniques used to quantify SAF in different matrices. These approaches include UV-visible spectrophotometric, spectrofluorimetric, electrochemical, and chromatographic techniques. Moreover, the biological sample pretreatment and extraction methods to reduce the potential impact of any matrix effects were highlighted in the present review, targeting eco-friendly extraction techniques. The advantages and disadvantages of each technique were outlined. As a future perspective of this review, eco-friendly microextraction techniques and other hyphenated techniques, such as capillary electrophoresis, have not yet been explored for SAF determination from pharmaceuticals or biological samples.

Keywords: Chromatography, Electrochemical, Safinamide mesylate, Spectrophotometric, Spectrofluorimetric.

1. Introduction

Parkinson's disease (PD) is a disease of the nervous system characterized by movement symptoms and the aberrant degeneration of dopamine-releasing neurons in the substantia nigra [1, 2]. The prevalence of PD doubled between 1990 and 2015. This steady increase leads forecasting models to estimate that fourteen million people will be affected by PD by the year 2040 [3]. For every patient, healthcare expenses might reach $20,000, while medication expenditures can be as high as $6,000 [4, 5]. The gold standard for treating PD is levodopa. However, issues including dyskinesia, stiffness, and motor abnormalities are common side effects of using levodopa for an extended period of time [6].

When PD patients experience these issues, their pharmaceutical regimen frequently includes additional medications. Examples of such medications include dopamine agonists, monoamine oxidase B (MAO-B) inhibitors, and catechol-O-methyl transferase inhibitors [7, 8]. SAF is considered one of MAO-B's most important reversible inhibitors [9]. SAF is nearly one thousand more selective for MAO-B than MAO-A in humans inhibiting dopamine metabolization, which increases dopamine levels in the brain [10]. In 2015, the European Medicines Agency first approved and registered SAF.

In the 1st trimester of 2017, SAF was approved by FDA, and two years later in 2019, SAF was authorized in Canada [11] and India [12]. SAF has the chemical formula (S)-2-((4-((3-Fluorobenzyl) oxy) benzyl) amino) propanamide methanesulfonate, and its structural formula is presented in **Figure 1** [13]. SAF is a crystalline powder that is white or off-white in color and has a melting point of approximately 210 °C. It is readily dissolvable in water, methanol, and dimethyl sulfoxide but sparingly soluble in ethanol and virtually insoluble in ethyl acetate. The main route of elimination for SAF *via* renal pathway in the urine, as it is oxidized to an inactivate metabolite. With a half-life of approximately 22 hours, SAF has a linear and dose-dependent absorption profile. At steady state, nevertheless, it is eliminated from the body without building up in the clinical setting [14].

Following a single dose, the highest concentration is attained at 2 hours; however, with repeated doses, the peak concentration is obtained at 5-6 hours. The plasma protein binding of this substance is about 89%. Interactions between medications can impact the safety or effectiveness of other drugs and alter the way the drug is absorbed into the body. Ketoconazole does not have a notable impact on the body's SAF levels. The simultaneous use of SAF with some antidepressant medications in patients with PD appeared to be both effective and well-

tolerated [15]. Nevertheless, many antiepileptic medications, such as phenobarbital and carbamazepine, have the effect of reducing the amount of SAF in the bloodstream and decreasing its duration of action [16–18]. However, SAF does not change the amounts of these drugs in the blood. It also doesn't change oral tyramine, which intestinal MAO-A mostly breaks down. SAF only stops MAO-B from working [19]. Several studies have shown that pain is a prevalent complaint in PD patients, with percentages ranging from 68% to 95% [20, 21].

In order to determine the best analgesic therapy for PD pain, a comprehensive review and meta-analysis were reported, where the options included safinamide, cannabinoids, opioids, painkillers, Chinese and electrical therapies, surgery, and pardoprunox [22]. Researchers have found that SAF directly relieves pain and makes people less likely to use painkillers [23]. The fact that it alleviates pain and other Parkinsonian symptoms further increases its appeal as an adjuvant treatment.

Safinamide's capacity to enhance various facets of PD patients' quality of life was also assessed. The mini-mental state examination, Parkinson's disease questionnaire, and Unified Parkinson's Disease Rating Scale part two were used to assess improvement. The Mini-Mental State Examination is a popular 30-item cognitive assessment instrument; in Parkinson's disease, it tracks the disease's course and the patient's reaction to treatment [24]. In clinical practice, Unified Parkinson's Disease Rating Scale Part Two is a standard assessment instrument for Parkinson's disease patients.

The patient's capacity to speak, dress themselves, and deglutition are among the everyday life activities evaluated [25].The 39-item Parkinson's Disease questionnaire (PDQ-39) is an all-encompassing evaluation that measures their capacity for paying attention, memory for the recent past, symptoms of depression, freedom of movement, and social support and relationships [26].

This review aims to outline the different analytical techniques used for SAF determination in pharmaceuticals or in biological samples, highlighting the advantages and disadvantages of each method alongside the most sensitive technique. Moreover, a short description of the techniques used for SAF extraction from different matrices mentions the most efficient extraction technique with the best recovery %.

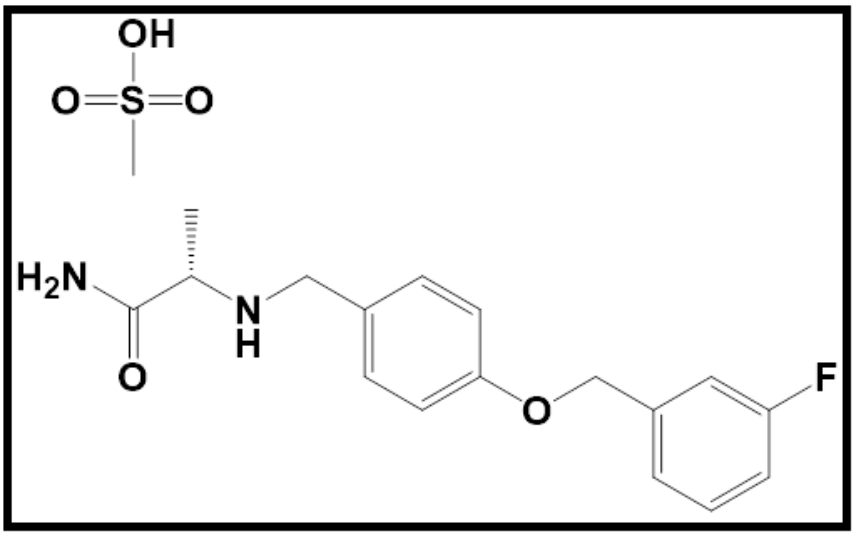

Figure.1. Chemical structure of safinamide mesylate

2. Sample pretreatment:

Sample pretreatment and SAF extraction reduce the potential impact of any matrix effects. An efficient extraction method is crucial to improve the sensitivity and reliability of analytical techniques. For SAF extraction from pharmaceuticals, a simple ultrasonic-assisted alcoholic extraction, followed by centrifugation, was the most used technique [27–29]. Different extraction protocols were implemented to extract SAF from biological samples efficiently. For example, coupling a deproteinization step and liquid-liquid extraction (LLE) exhibited satisfactory recovery (85.36%) as previously reported for SAF extraction from human plasma [30]. A high recovery (≈100%) of SAF from plasma was achieved *via* LLE using diethyl ether at pH 7.4 [31]. Another simple method displaying high SAF recovery (≈100%) is the deproteinization using different organic solvents such as acetonitrile and methanol, followed by centrifugation [14, 32]. A salt-aided deproteinization was also reported to enhance the recovery [29]. Internal standards, such as deuterium-labeled safinamide (safinamide-d3) [14] or diclofenac [30] may be added during extraction and analysis.

3. Analytical techniques

SAF is a non-official drug. However, a few approaches were reported for quantitation in pharmaceutical dosage forms or biological samples. Spectroscopic methods were mainly applied to pharmaceuticals, where the impact of the matrix interferences is lower than that in biological samples. However, hyphenated techniques, such as chromatographic ones, are generally used for biological samples.

a. Spectroscopic methods

Spectrophotometric method

Five spectrophotometric methods were reported to determine SAF in bulk powder and pharmaceutical formulation in the presence of its synthetic precursor, 4-hydroxybenzaldehyde. The spectrophotometric methods included: first derivative, derivative ratio, ratio difference, dual wavelength, and Fourier self-deconvolution[28]. All these methods were validated according to the ICH guidelines and exhibited comparable results regarding the explored validation parameters. However, the Fourier self-deconvolution method displayed slightly higher sensitivity with a limit of detection (LOD) of 0.6 µg/mL, and the derivative ratio method showed a fairly wider linear range (5-35 µg/mL). Although these spectrophotometric assays represent cost-effective and straightforward analytical techniques compared to chromatographic ones, they displayed a relatively narrow dynamic range (5-35 µg/mL) with lower sensitivity (LOD ≈ 1 µg/mL) [28].

Spectrofluorimetric method

As SAF possesses native fluorescence within excitation and emission wavelengths in the UV region, with a satisfactory quantum yield (≈ 45%). The spectrofluorimetric approach included detecting SAF emission at 299 nm upon excitation at 226 nm and utilizing methanol as a solvent. It was applied to a dosage form and spiked human plasma [32]. Even though this spectrofluorimetric analysis exhibited a wide dynamic range of 50 – 2000 ng/mL with high sensitivity in the low nanogram range (LOD ≈ 12 ng/mL) with high recovery (97-101%), it was only evaluated in spiked human plasma and not in real human plasma samples.

b. Electrochemical determination

Square wave voltammetry with chromium-doped zinc oxide nanoparticles as the working electrode has been reported to measure SAF in its dosage form and human plasma [29]. A high sensitivity in the sub-micromolar range (LOD = 0.4 µM) was achieved alongside a good SAF selectivity in the presence of its co-administered drug (L-Dopa) or co-formulated ingredients [29]. This voltametric method was only evaluated in spiked human plasma. However, the same shortcoming is that the proposed method is not extended to assess SAF in real human plasma samples.

Another reported eco-friendly method for SAF determination in its dosage form involved the utilization of a solid-contact potentiometric sensor that was modified with a Prussian blue analogue as an ion-selective electrode. This potentiometric technique revealed a wide dynamic range of 10^{-2} – 10^{-5} M with a good sensitivity in the low micromolar range (LOD = 9 µM). The

reported potentiometric sensor is only applied to pharmaceuticals and displays a high recovery (99.7 %) [27].

c. Chromatographic methods

Different chromatographic techniques were applied to estimate SAF in pharmaceuticals or in biological fluids. Liquid chromatography (HPTLC, HPLC, and UPLC) coupled to different detectors (UV-Vis absorbance, fluorescence, and MS) was the only hyphenated technique reported for SAF analysis, as shown in **Table 1**. These methods vary in sensitivity and their dynamic linear range. The highest sensitivity was achieved *via* tandem MS, displaying an LOD in the low picogram range (11.83 pg/mL) [33]. As the C_{max} reaches 411.5 ng/mL [34] after 50 mg single oral dose, the analytical method should accurately display sufficient sensitivity to quantify SAF in plasma in the low nanogram range. Therefore, for bioavailability, pharmacokinetic and toxicokinetic studies, analysis of SAF in biological fluids (i.e. plasma, urine and bile) was achieved *via* RP-HPLC coupled to sensitive detectors such as FL or MS. The LC/FLD resulted in a wide linear dynamic range (20-1000 ng/mL) with satisfactory recovery (84-92%) and a relatively lengthy analysis time (16 min). Whereas, the LC/MS displayed higher performance with wider linear range (20-20,000 ng/ml) alongside higher recovery (≈ 100%) within 5 min total analysis time [31].

For the analysis of SAF in pharmaceuticals, as shown in Table 1, many chromatographic analyses were reported for chiral purity assessment or stability indicating assays. SAF is an optically active compound, and it was previously reported that the (*S*)-enantiomer of SAF displayed a significantly higher selectivity and affinity towards MAO-B than its corresponding (*R*)-enantiomer [35]. The SAF drug is marketed as a single enantiomer *(S*)-alaninamide. However, it can be accompanied by traces of undesired (*R*)-enantiomer that can be present as an impurity and would show signs of toxicity at lower doses than the *S*-enantiomers. The chiral SAF purity testing was realized *via* enantioselective RP-HPLC coupled to a UV-Vis detector, revealing high sensitivity in the low nanogram range (15 ng/mL) [36].

For stability indicating assays, all the reported methods were based on RP-HPLC or RP-UPLC coupled to a UV-Vis detector. Another technique was reported to analyze SAF in the presence of its degradants and co-administered drugs (L-dopa & ondansetron). Whereas, RP-LC hyphenated to (ESI)MS/MS was used for the analysis of a potential genotoxic impurity (N-nitroso SAF), revealing high sensitivity (LOD 5 ng/mL) [37].

Table 1: **Chromatographic methods for the determination of Safinamide mesylate**

Stationary phase	Mobile phase/ Developing system	Detector	Application (Sample matrix)	Linear range & sensitivity	Ref
Chiralcel OD /Chiralpak AD RH (5 µm, 150 x 4.6 mm)	NaH_2PO_4 (300 mM, pH 3), CH_3OH, CH_3CN (65:25:10, v/v/v)	UV-Vis (220 nm)	SAF and R-enantiomer	Range 50-600 ng/mL LOD 15 ng/mL LOQ 50 ng/mL	[36]
HPTLC plates precoated with silica gel 60 F254	Toluene, CH_3OH, triethylamine (4: 1: 0.5, v/v/v)	UV-Vis (226 nm)	SAF in bulk and tablet dosage form	Range 400–2400 ng LOD 13.09 ng LOQ 39.67 ng	[38]
ODS-3 C18 column	0.1% aq. formic acid (pH 5) and CH_3OH Gradient elution	UV-Vis (220 nm)	SAF/ 4 impurities and 5 degradants	Range 50-1000 ng/mL LOD 27 ng/mL	[39]
UPLC CORTECS C18 (2.7 µm, 100 x 4.6 mm)	0.1% aq. formic acid and CH_3OH, (30:70, v/v) Isocratic elution 0.8 mL/min	(ESI)MS/ MS	SAF and SAF d4 in human plasma	Range 113-338 pg/mL LOD 11.83 pg/mL LOQ 35.84 pg/mL	[33]
Hypersil BDS C18 (5 µm, 250 x 4.6 mm)	CH_3OH and phosphate buffer pH 6.8 (80:20, v/v) Isocratic elution 1 ml/min t_r 5.1 min	UV-Vis (226 nm)	SAF and its degradation products	Range 40-180 µg/mL LOD 0.15 µg/mL LOQ 0.6 µg/mL	[40]
Primesil C18	CH_3CN & KH_2PO_4 (20 mM, pH 5), (40:60, v/v).	UV-Vis detector (226 nm)	SAF, its basic degradant, levodopa & ondansetron	Range 0.5–10 µg/mL LOD 0.11 µg/mL LOQ 0.33 µg/mL	[41]
NEOSPHERE RP C18	80% aq. CH_3OH Isocratic 1 mL/min t_r 5.2 min	UV-Vis (226 nm)	SAF in bulk and tablet	Range 5 -30 µg/mL LOD 0.27 µg/mL LOQ 0.83 µg/mL	[42]
HPTLC plates precoated with silica gel 60 F254	Toluene, CH_3OH, trimethylamine (4: 1: 0.5, v/v/v)	UV-Vis (226 nm)	SAF in bulk and tablet	Range 400-1200 ng/mL LOD 13.09 ng/mL LOQ 39.67 ng/mL	[42]
ACQUITY BEH C18 column	$(NH_4)_2HPO_4$ (20 mM, pH 9.0) and CH_3CN (80:20 v/v)	UV-Vis (272 nm)	SAF in presence of its degradation products	Range 10-60 µg/mL LOD 0.145µg/mL LOQ 0.4408 µg/mL	[43]
Kromasil C18 (5 µm, 250 x 4.6 mm)	Isocratic elution, 1 mL/min CH_3OH and 0.025% aq. CF_3COOH (45:55, v/v)	UV-Vis (226 nm)	SAF in presence of its degradation products	Range 10.00-150.00 µg/mL LOD 0.744 µg/ml LOQ 2.255 µg/ml	[11]
VDSpher PUR 100 C18-E	$HCOONH_4$/CH_3COONH_4 (20 mM, pH 5.5) and EtOH (60:40, v/v)	PDA (220 nm)	SAF in presence of precursor impurity	Range 4-100 µg/mL LOD 0.95 µg/mL LOQ 2.89 µg/mL	[44]
Acquity UPLC CSH C18 (1.7 µm, 2.1×100 mm)	CH_3COONH_4 (9 mM) and CH_3CN (22:78, v/v)	(+) ESI/MS (MRM)	SAF in human plasma	Range 0.1–1000 ng/mL LLOQ 0.1 ng/mL	[30]

Acquity UPLC C18	Gradient elution (0.1% formic acid- CH_3CN)	Mass detector	SAF in rat plasma	Range 1-2000 ng/mL LLOQ 20 ng/mL	[45]
Symmetry C18	CH_3OH and water (45:55, v/v)	UV-Vis (226 nm)	SAF in the presence of its degradation products	Range 24-120 µg/mL LOD 5.5 µg/mL LOQ 16.7 µg/mL	[46]
XBridge C18 (5 µm, 250 x 4.6 mm)	CH_3COONH_4 buffer (pH 5.8) / CH_3CN (55:45, v/v) Isocratic (1 mL/min) t_r 3.8 min	UV-Vis (226 nm)	bulk and in tablet dosage form	Range 10-60 µg/ml LOD 2.85 µg/ml LOQ 9.5µg/ml	[47]
CAPCELL PAK C18 MG II (3 µm, 50 × 2.0 mm)	0.1% aq. Formic acid/ 0.1% formic acid in acetonitrile. Isocratic (0.6 mL/min)	(+) ESI/MS	SAF in human plasma	Range 5.00–1000 ng/mL LOQ 5.0 ng/mL	[34]
Hypersil BDS C18 (5 µm, 150 x 4.6 mm)	50 mM phosphate buffer pH3-CH_3CN (68:32, v/v) Isocratic (0.5 mL/min) t_r 9.1 min	Fluorometric, $\lambda_{ex.}$ 224 nm; $\lambda_{em.}$ 302 nm	SAF in human plasma	Range 20−1000 ng/mL LOQ 20 ng/mL	[31]
Luna C18 (2), (3 µm, 75 x 46 mm)	CH_3COONH_4 (10 mM): CH_3CN (50:50, v/v) Isocratic (1 mL/min) t_r 0.9 min	(API) MS/MS	SAF in human plasma	(micro) biassay Range (0.5-20) 20-6000 ng/mL LOQ (0.5) 20 ng/mL	[31]

4. Conclusion

This article summarizes various analytical methods for quantifying SAF in pharmaceuticals and biological fluids. Based on the outcomes of this investigation, the chromatographic procedures were the most used, followed by spectroscopic and electrochemical approaches. Some offered approaches followed green chemistry guidelines and were assessed using various green evaluation criteria. Although hyphenated techniques, such as chromatographic ones, displayed the highest sensitivity in the low picogram range, these methods are sophisticated and require experienced personnel. Even though capillary electrophoresis offers a powerful hyphenated analytical technique for drug analysis, it's not yet explored for SAF determination. Moreover, eco-friendly extraction techniques such as solid phase microextraction or liquid-liquid microextraction are not investigated for SAF extraction and enrichment from biological samples, enhancing the recovery % and method sensitivity. Different reviewed extraction and analytical protocols displayed high recovery and sensitivity, but still have shortcomings, i.e., time-consuming, hazardous organic solvents, expensive materials, and instruments. Therefore, it is necessary to establish fast, eco-friendly, and cost-effective methods while ensuring

sensitivity. Such an approach shall aid the analyst in deciding on the best strategies to estimate SAF in different matrices.

Conflict of Interest

None of the authors found any conflicts of interest.

5. References

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