

Techniques for analytical estimation of COVID-19 clinical candidate, niclosamide in pharmaceutical and biomedical samples

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Niclosamide is a well-known broad-spectrum antiparasitic drug used for human as well as veterinary tapeworm infections. Recently, it attracted attention as an antiviral agent for treating coronavirus disease 2019. It is administered orally in humans to treat tapeworm infections. Furthermore, it is a registered pesticide and molluscicide to control infections in the aquaculture industry. Its chronic environmental exposure has potential toxicities when such contaminated seafood is consumed. Therefore, monitoring its residual concentration in food products (seafood, water, water waste, etc.) and pharmaceuticals (active pharmaceutical ingredients, bulk drugs, and formulations) is imperative. The present review critically investigates the sophisticated techniques employed for analyzing niclosamide, its degradation products, and metabolites in various samples and matrices. The future scope for green analytical methods, green sample extraction and preparation is also deliberated.

KEYWORDS

analytical techniques, antiviral, clinical application, niclosamide, pesticide

1 | INTRODUCTION

Niclosamide, 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide, is a Food and Drug Administration-approved anthelmintic drug with broad-spectrum antiviral properties [1]. World Health Organization has approved niclosamide as a molluscicide and has been used to control snails since 1960 [2]. For humans, it was launched in the USA under Bayer's brand name "niclocide," but later, it was withdrawn from the market. The developmental status of niclosamide in various countries is depicted in Figure 1. Besides the niclosamide-free base, its different salts/co-crystals or conjugates are also under active development. These

include CP-niclosamide, niclosamide ethanolamine, niclosamide sulfamate, and niclosamide-2-aminothiazole cocrystal. The CP-niclosamide is a peptide-drug conjugate comprising two molecules of niclosamide linked to an elastin-like polypeptide. It is in the preclinical stage of development by Duke University as an anticancer drug [3]. Ethanolamine salt of niclosamide is also investigated at the preclinical stage as an anticancer drug and for treating Type 2 diabetes by Howard Hughes Medicinal Institute [4]. Ohio State University performed and reported the biological testing of a prodrug, niclosamide sulfamate, for cancer hormone therapy [5]. Additionally, cocrystals of niclosamide with 2-aminothiazole were prepared by Westfaelische Wilhelms-University, Münster [6]. The developmental status of niclosamide depicted in Figure 1 signifies its importance in treating various therapeutic indications such as helminthic infections [7], cancer [8], and viral infections [9, 10]. In recent years, niclosamide

Article Related Abbreviations: API, active pharmaceutical ingredient; LC-PDA, liquid chromatography-photodiode array; PGE, pencil graphite electrode.

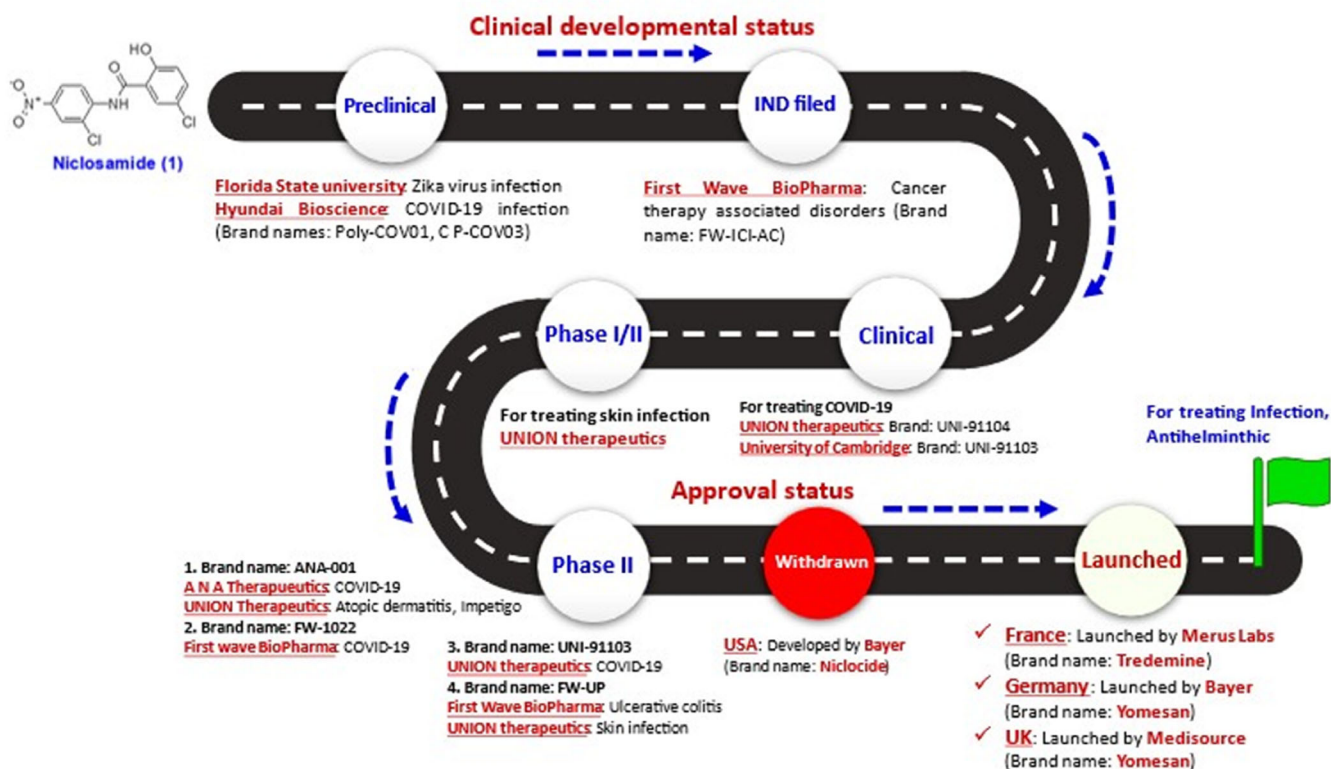


FIGURE 1 Roadmap of the current development status of niclosamide.

attracted attention as an antiviral agent for treating coronavirus disease 2019 (COVID-19). There are 19 clinical trials underway (11 studies completed/terminated) to identify the potential of niclosamide in treating COVID-19. Niclosamide inhibits the replication of severe acute respiratory syndrome coronavirus 2 via a pH-dependent endocytic pathway. It is an endosomal acidification inhibitor wherein, due to the protonophore activity, it neutralizes endosomal acidic pH, which is crucial for viral replication [11].

We conducted a PubMed search with the keyword 'niclosamide' focusing on 'review' articles which yielded 87-hits from 1967 to 2022. The highest number of reviews were published in 2020 > 2021 > 2019 with 18 > 16 > 12 number of articles, respectively. Most of the reviews are related to the drug's pharmacological effects with no precedence of a review on analytical techniques. Many reviews discuss its use in treating therapeutic indications [1]. A couple of reviews unravel its mechanism of action [11], various drug deliveries [12], and biological, clinical, and epidemiological features of COVID-19 [13]. Few reviews also discuss the molecular mechanism of niclosamide in colorectal cancer [14], prostate cancer [15], and renal cell carcinoma [16]. The biology and toxicology of the drug were also reviewed. Niclosamide, as an antiviral [9, 17, 18], anthelmintic [19–23], antiparasitic [24], and mollusci-

cides [25, 26], was also reviewed. Despite the discovery of niclosamide in the 1960s, the analytical methods for estimating niclosamide have never been reviewed. Hence, this review aims to provide a detailed account of the analytical techniques used for analyzing this important drug in pharmaceuticals, environments, and biological samples. The literature search was conducted using PubMed-NCBI, Reaxys, SciFinder, Clarivate: Drug research advisor, and so forth.

2 | DISTRIBUTION OF ANALYTICAL TECHNIQUES

The spectrum of analytical data obtained on niclosamide is presented in Figure 2. We obtained ~87 literature reports with the keywords 'niclosamide' and 'analytical methods'. For ease of understanding, this literature was segregated into two categories, namely i) method category and matrix analyzed, and ii) analytical techniques.

The analytical methods for niclosamide were categorized according to its application, such as the component analyzed. These include active pharmaceutical ingredient (API) and metabolites, food, water, wastewater and sludge, pesticides, toxins, bioassay, forensic, soil, environmental, isotope, and organic contaminant analysis. The detailed

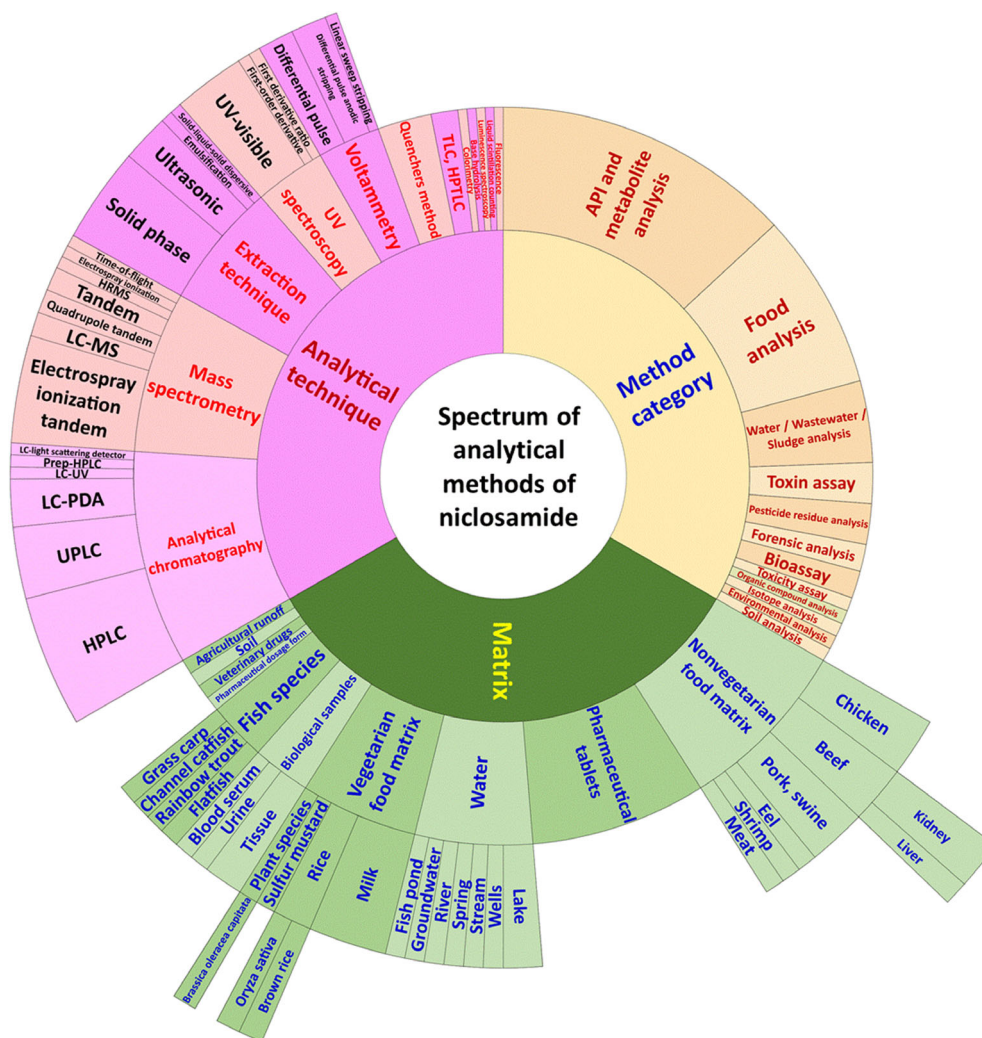


FIGURE 2 Overview of analytical methods and matrices for niclosamide analysis

analysis depicted maximum analytical methods reported for API and metabolite analysis > food analysis > water/wastewater/ sludge (~39 vs. 23 vs. 11 %).

Various analytical techniques are reported to analyze niclosamide in pharmaceuticals, food, and environmental samples. These methods were further categorized according to the advanced/specialized instruments. The major sophisticated technologies in analyzing niclosamide include chromatography, mass spectrometry, and voltammetry. The chromatographic techniques, including HPLC, UPLC, prep-HPLC, LC-photodiode array (LC-PDA), and LC-UV dominated other methods with ~28% contribution. Mass spectrometry ranked second in the sophisticated techniques (~21%), including electrospray ionization, quadrupole, LC-MS, time-of-flight, high-resolution mass spectrometry, and so forth. UV spectroscopy and voltammetry contributed to ~9.4% and 8.2%, respectively. The other minor analytical techniques include the quenchers method, TLC, HPTLC, fluorescence, liquid

scintillation counting, luminescence spectroscopy, base hydrolysis, colorimetry, and so forth. These are discussed in the subsequent sections.

The diverse sophisticated methods are reported to analyze niclosamide and its metabolites. The low bioavailability of niclosamide may be attributed to its rapid first-pass metabolism in the intestine and liver (Figure 3). Phase I drug metabolism comprises oxidation, reduction, and hydrolysis reactions to convert drugs into polar molecules. The phase I metabolites of niclosamide are 2',5-dichloro-4'-aminosalicylanilide, and 3-hydroxy niclosamide [27]. The polar metabolites undergo phase II metabolism such as glucuronidation, acylation, and sulfation to yield niclosamide-O-glucuronide [28, 29]. Hence, sophisticated techniques such as MS/MS are ideal for identifying and monitoring mass, retention time, and fragmentation spectrum.

Niclosamide was approved in 1964 by the US Environmental Protection Agency as a lampricide and mollusci-

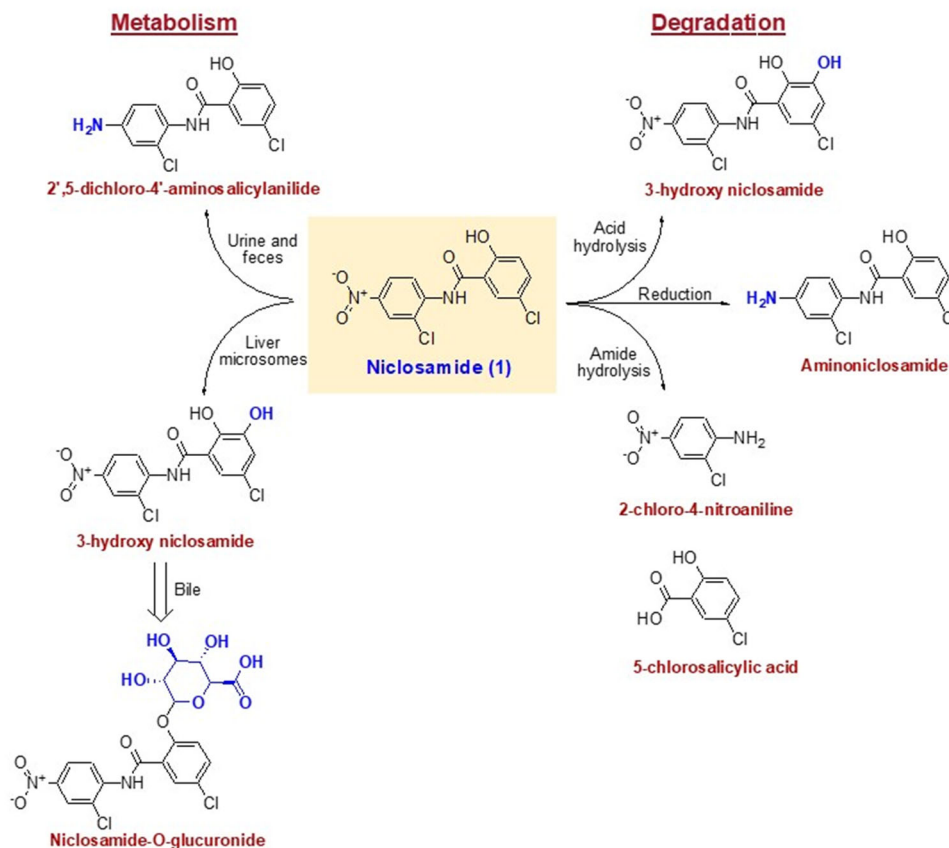


FIGURE 3 Metabolites and degradation products of niclosamide

cide. It controls sea lamprey larvae and freshwater snails that carry the vectors for diseases that affect humans. It is classified as a toxicity category III molecule which is slightly toxic and irritating. It is also a registered pesticide, so it is imperative to determine its residual concentration in food products. Various analytical methods are reported to monitor the concentration of niclosamide in aquatic animal tissue, water, and rice. Furthermore, as an environmental pollutant, niclosamide concentration is also estimated in water, wastewater, sludge, and soil samples. Apart from sophisticated analytical techniques, various extraction methods are also reported for detecting pesticide residue in environmental samples.

3 | ANALYTICAL METHODS FOR ESTIMATION OF NICLOSAMIDE

Thorough literature research revealed several analytical techniques employed in qualitative or quantitative analysis of niclosamide in matrices. As depicted in Figure 2, the analytical methods are divided based on method category, matrices to be analyzed, and analytical techniques. They are discussed in the following sections.

3.1 | Classification based on method category/matrix analyzed

3.1.1 | API and metabolite analysis

This method category includes evaluating forced degradation products, namely 5-chlorosalicylic acid and 2-chloro-4-nitroaniline, pharmaceutical preparations including tablets, and quantitative estimation of niclosamide post-derivatization to amino-niclosamide, bulk, and synthetic mixture analysis.

3.1.2 | Food analysis

Niclosamide is an approved molluscicide used on a large scale in aquacultures. It is critical to determine its residual concentrations in food such as muscle tissues of rainbow trout, channel catfish, water, rice crops, and soil. It is a potentially toxic agent for aquatic animals and can cause DNA damage [30, 31]. Therefore, its recovery study from animal tissue is essential. The maximum residue level as per Australian standards is 0.01 mg/kg [32]. Bayluscide contains niclosamide ethanolamine, a registered

molluscicide for controlling sea lamprey populations [33]. Few reports are available to estimate the concentration of residual niclosamide in the muscle fillet tissue of fish.

3.1.3 | Water, wastewater, soil, and sludge analysis

There are degradation products of niclosamide reported in the literature. The acid hydrolysis yields hydroxyniclosamide. Aminoniclosamide is obtained in the presence of reducing agents and light [34]. Additionally, amide hydrolysis of niclosamide yields 2-chloro-4-nitroaniline and 5-chlorosalicylic acid [27, 35]. A continuous connection exists between surface and underground water in flooded crops such as rice. This may lead to the entry of mobile chemicals, including pesticides such as niclosamide which pose a risk to humans where underground water is used for drinking [36]. Hence, environmental monitoring of these trace contaminants is crucial, and there are a couple of sophisticated techniques to analyze niclosamide and its degradation products in water, wastewater, and sludge [32, 36].

3.1.4 | Forensic analysis

The fishery industry is vital in building up some countries' regional and national economies. Various agrochemicals, for instance, niclosamide, pentachlorophenol, and fenprothrin may be used with ill intentions to eliminate economic competition [37, 38]. Identification of poison is essential in forensic sciences. Generally, the GC-MS technique is used to identify poisons; however, the agrochemicals mentioned herein are medium to high polar chemicals challenging to detect by GC-MS. Therefore, in 2013, researchers from the Wuhan Institute of Technology developed the LC-MS/MS method for the simultaneous estimation of three agrochemicals mentioned above. Pentachlorophenol was determined using selective ion monitoring mode, whereas niclosamide and fenprothrin were estimated using multiple reaction monitoring mode.

3.1.5 | Matrix analyzed

Various matrices analyzed include pharmaceutical preparations (bulk drugs and tablets) and nonvegetarian food matrices such as chicken, beef, pork, swine, eel, shrimp, meat, and so forth. Besides this, water is also a major source of pesticide contamination; therefore, the residual pesticide analysis in fish pond water, groundwater, river, spring, stream, wells, and lake water is also critical. The vegetar-

ian food matrix includes milk, rice, sulfur mustard, and plant species. The drug and metabolite analysis in biological samples need special extraction procedures considered biological samples. These include analysis of blood serum, urine, and tissue. The trace amounts of niclosamide, degradation products, and metabolites may be present in aquatic products such as various fish species. These species include grass carp, channel catfish, rainbow trout, flatfish, and so forth. The multiple matrices which may be analyzed are agricultural runoff, soil, and veterinary drugs. The matrices mentioned in this section must be effectively extracted for niclosamide using special extraction technique/s coupled with an analytical method for quantitative estimation. The maximum number of methods reported are for pharmaceutical preparation > nonvegetarian food matrix > water samples > vegetarian food matrix.

3.2 | Analytical techniques employed for estimation of niclosamide and its metabolites

The techniques reported for the analysis of niclosamide are presented in Figure 2. These analytical techniques are discussed in the following section.

3.2.1 | Analytical chromatography

Chromatography is a sophisticated technique for analyzing drugs in various matrices such as APIs, formulations, biological samples, and so forth. The analytical chromatographic methods covered in this section for analyzing niclosamide are HPLC, UPLC, LC-PDA, LC-UV, preparative-HPLC, and LC-light scattering detector. There are 11 HPLC-based methods [30, 32, 36, 37, 39–45] with various detectors for analyzing niclosamide, summarized in Table 1.

Mass spectrometry is one of the powerful quantitative analytical tools which measures the mass to charge ratio (m/z) of the analyte present in the samples. Several methods for analyzing niclosamide use the MS tool to detect an analyte, combined with physical separation capabilities and analyzers.

The electrical energy assists the transfer of ions from the solution into the gaseous phase, which is further subjected to MS analysis. ESI involves the generation of fine spray wherein, upon evaporation of the solvent, the ions are ejected and further measured. Doran and Stevens determined niclosamide and its degradation products in water using LC-MS/MS. The authors compared the sensitivity and separation of degradation products, 2-chloro-4-nitroaniline, aminoniclosamide, hydroxyniclosamide, and

TABLE 1 Chromatographic methods for estimation of niclosamide in formulations and biological samples

Sr. No.	Sample	Method details	Sample extraction	Detector	Linearity range	LOQ	LOD	Reference
1	Degradation products	Kinetex XB-C18 column (75 × 4.6 mm × 2.6 µm); column temp. of 35°C Mobile phase: Water (Phase A) and Methanol (Phase B), all containing 0.2% formic acid (v/v) with a flow rate of 0.5 ml/min Gradient elution: Time, A:B (%v/v) = 0→1 min, 90:10; 6→8 min, 20:80; 8.5→10.5 min, 10:90; 11→13 min, 90:10.	NA	DAD (330 nm) and 6410 quadrupole tandem mass spectrometer	Niclosamide: 1–100 ng/ml 2-chloro-4-nitroaniline: 50–2200 ng/ml Aminoniclosamide: 2–2500 ng/ml; Hydroxyniclosamide: 2–300 ng/ml; 5-chlorosalicylic acid: 5–1000 ng/ml	0.1 ng/ml	NA	[36]
2	Degradation products from pharmaceutical tablets	Shimpack column VPODS (25 × 4.6 mm) Mobile phase: methanol: water (70:30 v/v) at 1 ml/min	NA	UV detection at 320 nm	NA	NA	NA	[39]
3	Residue analysis in water, soil, and rice samples	UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 µm), maintained at 30°C. Mobile phase: A, water and B, methanol at 0.3 ml/min Gradient elution: Time (min)/ A (%) / B (%) = 0/90/10; 1.5/10/90; 3.5/10/90; 3.6/90/10; 5/90/10.	ACN	MS/MS	0.005–0.05 mg/kg	5 µg/kg	0.01 µg/kg	[32]
4	Aquatic animal tissue	Thermo Hypersil BDS octadecylsilane column (150 mm × 2.1 mm, 5 µm) at 35°C. Mobile phase: A, ACN, and B, water at a rate of 0.2 ml/min Gradient elution: 0–2 min, 50% A; 2–2.5 min, 50%→90% A; 2.5–7 min, 90% A, 7–7.1 min, 90%→50% A; 7.1–8 min, 50% A	Ammoniated ACN	ESI-MS-MS	0.5–100 µg/kg	0.5 µg/kg	0.2 µg/kg	[30]

(Continues)

TABLE 1 (Continued)

Sr. No.	Sample	Method details	Sample extraction	Detector	Linearity range	LOQ	LOD	Reference
5	Bulk API	Hibar C18 column (250 mm × 4.66 mm, 5 μm) Mobile phase: MeOH: (NH ₄) ₃ PO ₄ buffer (85:15 v/v) with pH adjusted to 5.47 with a flow rate of 1.2 ml/min	MeOH	PDA at 332 nm	0.01–100 μg/ml	0.01 μg/ml	0.0048 μg/ml	[40]
6	Degradation products	Fortis C18 column (150 × 4.6 mm, 5 μm) at 25°C Mobile phase: 0.1% formic acid and ACN at 1 ml/min Gradient elution: 0–6 min, 45% ACN; 6–8 min, 45%–90% ACN, 10–12 min, 45% ACN	NA	PDA at 334 nm	30–70 μg/ml	0.06 μg/ml	0.02 μg/ml	[41]
7	Tablets	Zorbax C18 (250 × 4.6, 5 μm) column at 30°C Mobile phase: KH ₂ PO ₄ : ACN (60:40 v/v) at 1 ml/min	MeOH	PDA at 290 nm	NA	NA	NA	[42]
8	Veterinary formulations	Luna C-18 (5 μm × 25 cm) column Mobile phase: ACN: buffer solutions (2:8, v/v) at 0.8 ml/min Buffer solution A: 0.005 M Na hexanesulphonic acid and glacial acetic acid (8 ml) and adjust the pH to 3 by phosphoric acid Buffer solution B: 0.01 M dibutylamine and adjust to pH 3 with phosphoric acid	<i>n</i> -propanol	PDA at 240 nm	10–180 μg/ml	NA	NA	[43]

(Continues)

TABLE 1 (Continued)

Sr. No.	Sample	Method details	Sample extraction	Detector	Linearity range	LOQ	LOD	Reference
9	Food analysis (fish sample)	Phenomenex Prodigy ODS reverse phase column (150 mm × 4.6 mm, 5 µm) Mobile phase: A, 58 mM sodium acetate buffer and B, ACN at 1 ml/min Gradient elution: 0–5 min, 35% A; 5–10 min, 50% A; 10–20 min, 60% A; 20–25 min, 80% A; 25–30 min, 100% A	Acetone extractions with clean-up on SPE	PDA at 360 and 335 nm	NA	NA	0.0107 µg/g	[44]
10	Pharmaceutical suspension	Phenomenex L1 HPLC analytical C18 100 Å ⁰ column (250 × 4.6 mm, 5 µ) Mobile phase: KH ₂ PO ₄ buffer + ACN (70:30 v/v) at 1.0 ml/min	0.1 N methanolic HCl	UV detection at 290 nm	80–130 µg/ml	9.936 µg/ml	5.673 µg/ml	[45]
11	Forensic investigations	Acclaim 120-C18 column (150 mm × 3 mm, 3 µm) at 30°C Mobile phase: A, 20 mmol/L ammonium acetate of pH 4.5 and B, MeOH at 0.3 ml/min Gradient elution: 0–4 min, 85% B; 4–13 min, 85%→90% B; 13–14 min, 90% B; 14–15 min, 90%→85% B; 15–19 min, 85% B	DCM: MeOH (4:1 v/v)	MS-MS	0.04–2 ng/ml	NA	0.02 ng/ml	[37]

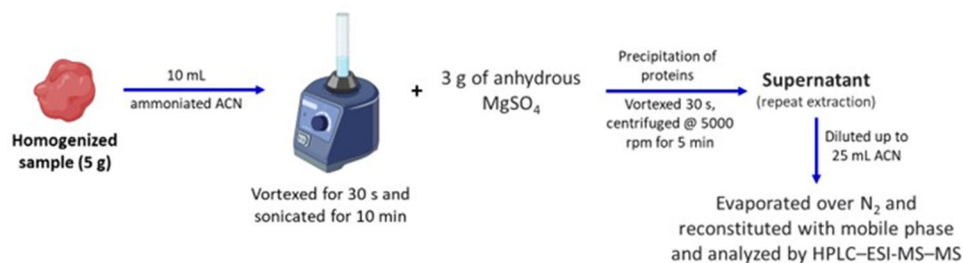


FIGURE 4 Sample extraction and preparation for analyzing niclosamide from aquatic tissues [30]

5-chlorosalicylic acid using MeOH and ACN. Methanol was 3–50 times better than ACN with LLoQs for all degradation products. The additional advantages of better sensitivity in MeOH include low flow rate, reduced solvent consumption, and cost. The reported method had 20 times lower LOQ than the previously reported methods, eliminating time-consuming clean-up [36]. The MS detection methods are also listed in Table 1 and have advantages over UV/PDA detection because they are QuEChERS.

3.2.2 | Sample extraction techniques

Sample preparation is very crucial in quantifying analytes in biological fluids. The sample may be processed in two ways: solid phase extraction and liquid-liquid extraction. To measure contamination of niclosamide in aquatic animals and edible tissues, GC, HPLC, and LC-MS/MS methods were reported. These methods require complicated, time-consuming sample preparations. Liu and coworkers developed a novel procedure for extracting niclosamide (Figure 4) from aquatic tissue samples, analyzed by HPLC-heated ESI MS/MS [30].

Niclosamide is one of the agrochemicals commonly used as molluscicides; however, it may be used with criminal intentions for poisoning aquaculture products. Jiang and coworkers developed the LC-MS/MS method to simultaneously estimate three toxicants: pentachlorophenol, niclosamide, and fenpropathrin in fishpond water samples. The agrochemicals were extracted with 10 ml of dichloromethane:acetone (4:1 v/v). The analysis was performed in selective ion monitoring mode with excellent reliability [37]. In 2018, Zhao et al. reported the LC-MS/MS method to estimate veterinary drugs of multiple classes, including tetracyclines and beta-lactams in meat products. The method reports 2-step solid-liquid extraction followed by efficient sample clean-up with EMR-lipid cartridges post-extraction. Extraction for multi-class drugs is challenging; hence two categories of sample preparation are reported. Category 1 is less selective, involving the use of ACN or ACN/water mixtures following clean-up techniques that lack sensitivity. On the

contrary, category 2 is used to extract complex matrix analytes. EMR-lipid sorbents interact with hydrocarbon chains of unbranched lipids, thus leaving the analyte in the solution for further analysis using LC-MS/MS. The method was also validated with porcine and bovine muscle, bovine liver, bovine kidney, and chicken liver [46]. Boontongto and coworkers developed a green analytical method involving ultrasound-assisted microextraction followed by centrifugation to efficiently extract anthelmintics in milk formulae. The analyte was extracted using surfactants: tween 20, tween 80, triton X-114, Triton X-100, SDS-CTAB, genapol X-080, and tergitol TMN-6. Among all these, genapol X-080 and tergitol TMN-6 were non-ionic surfactants with low UV absorption. In addition to these surfactants, the method replaced halogenated solvents, *viz.* chloroform, chlorobenzene, dichloromethane, and carbon tetrachloride, which are hazardous to handle with less toxic solvents such as cyclohexane, butanone [47]. The various method involving MS detection are listed in Table 1.

3.2.3 | Voltammetry

Voltammetry is a low-cost, sensitive, and selective electrochemical method for determining an analyte in the samples. In 2014, Dede et al. developed a voltammetric method using a pencil graphite electrode (PGE). The voltammograms of niclosamide were recorded using PGE at pH 7.0 in a Britton-Robinson buffer solution containing 0.1 M KCl and a 30% dimethylformamide mixture. The PGE displayed better electrochemical behavior than a glassy carbon electrode. The reported method was successfully used for determining niclosamide in tablets with no excipient interference [48]. Yao et al. reported differential pulse stripping voltammetric determination, which used carbon nanomaterials instead of glassy carbon electrodes. As an electrode, the authors reported one-dimensional carbon nanotubes and two-dimensional carbon material, graphene. Carbon nanomaterials are the best sensing material with high electrical conductivity and electrocatalytic activity. It has wide electrochemical

and biochemical applications and provided satisfactory results for the trace analysis of niclosamide in agricultural products [49].

3.2.4 | Miscellaneous methods

There are also a couple of methods such as colorimetry and base hydrolysis, liquid scintillation counting, and fluorescence to determine niclosamide in various samples. Paghadar and Vadia reported HPLC and HPTLC methods for analyzing niclosamide in bulk API and synthetic mixtures. The HPTLC method employed a TLC plate with silica gel G60 F254 with toluene: ethyl acetate (7:3 v/v) as the mobile phase. The HPTLC method was simple, specific, sensitive, and stability-indicating, which can be used for quantitatively estimating niclosamide in QC-QA laboratories. The linearity was found to be 200–700 ng/band. The LOD and LOQ reported were 36.21 ng/band and 109.7 ng/band, respectively [40]. In 2012, a fluorescence-based derivatization reaction was reported by Algarra et al. for the estimation of niclosamide. The non-fluorescent niclosamide is subjected to selective catalytic reduction to aminoniclosamide under a hydrogen atmosphere in the presence of MeOH: ethyl acetate (1:3) using Pd/C for three hours at room temperature. The aminoniclosamide exhibits green fluorescence at 439 nm, which is enhanced by forming an inclusion complex with methyl β -cyclodextrin [50].

4 | THE PERSPECTIVE AND CONCLUDING REMARKS

Niclosamide is a World Health Organization-approved molluscicide used to control freshwater snails. It is also a registered pesticide in 1964 in the US in the agricultural sector. Additionally, it is an oral anthelmintic drug used against tapeworm infections in humans. In the last 2–3 years, it fetched attention due to its antiviral properties for treating COVID-19 and is being investigated in many clinical trials. The SAR of a molecule is also modified in many drug discovery programs to identify more potent and druggable derivatives of niclosamide. During this process, analytical method development is critical; therefore, this review aims to provide insights on analytical techniques reported for this important molecule. The literature reports on analytical techniques may be extended for the synthetic derivatives of niclosamide during drug discovery and development.

Upon critically analyzing the reported methods for the analysis of niclosamide revealed that a) ~39% of methods involve analyzing API and its metabolites either

in formulations or biological samples, b) ~23% of methods involve analysis of food samples (aquatic tissues), and c) ~38% methods perform an environmental analysis of niclosamide in variety of matrices namely water, water waste, sludge, pesticide residue, toxins, forensic and soil samples. Due to its wide application, it is crucial to determine residual concentration analysis of niclosamide in the environment.

After careful investigation, it was found that in the mobile phase, methanol was used up to 85%. On the other hand, ACN was only used up to 40% v/v. The organic solvents were used in combination with aqueous buffers. Methanol and ethanol are biosolvents, but ACN, ethyl acetate, and THF are not; this may cause environmental hazards. In analytical methods, toluene and n-hexane may be replaced with D-limonene, terpenes, and so forth. [51]. According to the United States Environmental Protection Agency, ACN, and TFA are hazardous solvents. TFA is cytotoxic, corrosive, and persistent in the environment. While developing analytical methods for niclosamide, the usage of such toxic and hazardous solvents may be restricted, and these may be replaced with biosolvents in a suitable combination with aqueous buffers. Additionally, organic solvents may also be replaced with supercritical solvents such as CO₂. The advantage of supercritical fluids is the efficient extraction of the components as the polarity is comparable to cyclohexane, which facilitates the effective extraction of the nonpolar analyte [52, 53]. One colorimetric biobased method for analyzing niclosamide wherein naturally occurring methyl β -cyclodextrin is used as a complexation reagent, which enhances the formation of colored complex [50].

Green miniaturized sample preparation is preferred, wherein environmentally friendly extraction phases are used [54]. Additionally, microextraction is also preferred over traditionally used extraction procedures. Carbon nanotubes were also used as sorbents by Yao et al. to extract niclosamide in agricultural samples. Carbon nanotubes offer many advantages and high extraction capacity [49, 55]. Traditional extraction procedures require a large sample and solvent volume, pre-concentration followed by clean-up. On the contrary, microextraction is the preferred eco-scale technique due to in situ sample preparation which minimizes solvents and reagents. This eventually avoids sample derivatization and minimizes waste, sample, chemical, and material quantities [56–58]. Liquid phase microextraction with green solvents such as water, 1-propanol, ethanol, methanol, acetone, and so forth, may also reduce clean-up with efficient extraction of the analyte. The analytical methods for niclosamide mentioned in Table 1 use green solvents, namely a mixture of buffers with organic solvents, n-propanol, ethanol, acetone, and methanol [59].

It is imperative to analyze the drug and its metabolites in various matrices using a sensitive, precise, accurate, miniaturized, high-performance, stability-indicating analytical method. While developing an analytical method, green solvent, and miniaturized sample preparation must be considered. The available methods for analyzing niclosamide, its degradation products, and metabolites comprise chromatographic estimation followed by mass spectroscopic analysis. Despite these sophisticated methods, colorimetric analysis in the presence of cyclodextrin favors the analysis of niclosamide in agricultural waste. The utility of carbon nanotubes and graphene as sorbent material is also highlighted. Electrochemical methods are also available, which enable niclosamide estimation without sample destruction. Ultimately, selecting green solvents and extraction procedures to determine environmental pollutants is advantageous over traditional methods. This information will be valuable to scientists with analytical, medicinal, and formulation backgrounds working on these scaffolds or drugs.


CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Gupta DS, Bharate SS. Techniques for analytical estimation of COVID-19 clinical candidate, niclosamide in pharmaceutical and biomedical samples. *Sep Sci plus.* 2022;1–13. <https://doi.org/10.1002/sscp.202200097>