

## Deep eutectic solvent based method for analysis of Niclosamide in pharmaceutical and wastewater samples – a green analytical chemistry approach

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**Abstract:** The paper presents a simple, but very effective and sensitive spectrophotometric method for trace analysis of Niclosamide based on liquid-liquid microextraction using deep eutectic solvents (DESs) prior to its quantification. Here, different DES systems, such as Choline chloride (ChCl) + Urea, ChCl + Citric acid, ChCl + Ethylene glycol and ChCl + Phenol, were synthesized and evaluated at different molar ratios, selecting ChCl + Phenol 1:2 as an extractive DES system. Optimization studies revealed that best performance were obtained at pH 8 with optimum volume of THF and DES as 0.3 mL and 0.4 mL, respectively. The developed method is characterized by good analytical performance, e.g., a recovery of 99.26% and precision described by RSD value as <2%. The inter-assay precision was 0.51% while intermediate precision was 0.0323%. The method was found linear from 4.8 to 48 µg/L. LOD and LOQ were found as 0.112 and 0.374 µg/L, respectively. The paper presents also examples of the application of the proposed method for the determination of Niclosamide in different pharmaceutical and wastewater samples. This alternative method reveals a better performance in respect to the British pharmacopoeia procedure, providing concurrently ease of operation and simplicity.

**Keywords:** extraction, deep eutectic solvents, drugs, sample preparation, spectroscopy

## Introduction

Niclosamide is an important anthelmintic drug. This chemical is effectively used for killing beef, draft and dog tapeworms (Algarra et al., 2012). IUPAC name of Niclosamide is 5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide and its chemical structure is represented in Figure 1. Niclosamide is used as an antibacterial agent for the treatment of colitis and diarrhea. Furthermore, it is also effective for the treatment of parasites that infest humans, such as *Taenia solium*, *Diphyllobothrium latum* and *Hymenolepis nana* and threadworms. Recently, researchers have claimed that Niclosamide can act as an anticancer agent. It has also been reported that Niclosamide prevents the  $\beta$ -catenin, mTORC1, STAT3, NF- $\kappa$ B as well as Notch signaling pathways, and persuade cell cycle arrest, growth reticence and apoptosis (Shantier et al., 2015).

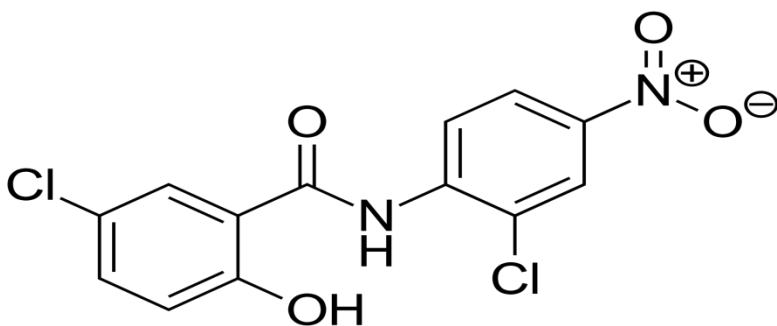


Figure 1: Niclosamide structure

Niclosamide inhibits the transcript and DNA binding ability of NF- $\kappa$ B. For instance, some investigations state that Niclosamide inhibits the NF- $\kappa$ B trail and increase ROS level to convince apoptosis in AML cells (Jin et al., 2010). Despite the above applications, Niclosamide is toxic and its accumulation negatively affects terrestrial and aquatic organisms. To date, the mechanism of action is not clearly explained; however, it has been discovered that Niclosamide displays a drastic effect on the respiration and metabolism of carbohydrates (Andrews et al., 1982, Lardans and Dissous, 1998, Oliveira-Filho and Paumgarten, 2000). Niclosamide, which has potent antiviral activity against single-stranded RNA viruses including coronaviruses, was proposed as an antiviral during the Severe Acute Respiratory Syndrome (SARS) outbreak in 2002; and more importantly, it has activity against Coronavirus 2 (SARS-CoV-2), inhibiting it *in vitro* studies, together with some other similar structured RNA viruses in both *in vitro* and *in vivo* assays (Wu et al., 2004).

Niclosamide is used for the treatment of parasitic diseases in fish as a bath additive or mixed with the feed. To some extent, it is effective in a narrow concentration range of 0.075-0.1 mg/mL (90 min)(Doran and Stevens, 2014); unfortunately, Niclosamide initiates to be toxic to all fish species at 0.5 mg/L (48 hours). Therefore, the U.S. Environmental Protection Agency (EPA) restricted the use of Niclosamide as a pesticide (Pohanish, 2014). On the other hand, higher concentration of Niclosamide may have very broad clinical applications against cancer, bacterial and viral infections, metabolic diseases, such as Type II diabetes, NASH and NAFLD, artery constriction, endometriosis, neuropathic pain, rheumatoid arthritis, sclerodermatous graft-versus-host disease, and systemic sclerosis (Chen et al., 2018). It is on the World Health Organization's list of essential medicines (Organization, 2019); however, at high concentrations, it may cause health issues. In water and sediment samples, Niclosamide is usually found in a concentration less than 1  $\mu\text{g/mL}$ , while several reports documented that Niclosamide has been determined in a wide range of concentration levels from 15  $\mu\text{g/mL}$  to 1000  $\mu\text{g/mL}$  (Alemu et al., 2003, Shah et al., 2016, Paghadar and Vadia, 2019, Muir and Grift, 1980, Doran and Stevens, 2014). Due to these reasons, the presence of Niclosamide in the environment must be monitored. Until, now, there are few analytical methods available for Niclosamide analysis including spectrophotometric (Daabees, 2000), chromatographic (Schreier et al., 2000), electrochemical (Sridevi and Reddy, 1991) as well as voltammetric methods (Pandey and Dugar, 2001), in which their low sensitivity is identified as the main drawback.

To the best of our knowledge, there is no single method available for the pre-concentration of Niclosamide. Few methods are available for Niclosamide analysis including HPTLC, HPLC (Paghadar and Vadia, 2015), reversed-phase HPLC and derivatization-based spectrophotometric method (Shah et al., 2016), differential scanning calorimetry (Al-Hadiya, 2005) and voltammetric method (Zhang et al., 2017). These methods are based on more complicated instrumentation including GC-MS; and the usage of more toxic chemicals (such as dichloromethane, carbon disulfide, tetrahydrofurane used as sample solvent used in GC and large quantities of acetonitrile and methanol in HPLC) in case of chromatographic methods (Dogan and Tobiszewski, 2020, Płotka-Wasyłka and Namieśnik, 2019). In HPLC methods, the solvent consumption exceeds 100 mL including solvents for analysis and reconditioning stage prior to the next run (Shah et al., 2016). While in this new method total consumption of solvent is 12 mL per analysis. In electroanalytical methods, multi-step modification of electrodes is needed (Alemu et al., 2003). At this point, spectroscopic methods are the easiest ones used for analysis. However, they are associated with matrix effects and do not offer a satisfactory detection limit.



Considering the major disadvantages of the available methods, this paper presents an attempt to develop a pre-concentration procedure prior to Niclosamide analysis that provides sufficient selectivity of extraction. This sensitive spectrophotometric method is low-cost and more robust compared to alternative HPLC and GC-based methods. In this study, deep eutectic solvents (DESs), as a novel “green” class of extraction medium, were used. Their usefulness has been proved for several applications dedicated to analytical methods, as well as process scale extraction towards many groups of analytes (Makoś et al., 2018, Makoś and Boczkaj, 2019, Haq et al., 2021). The purpose of this paper was to develop a selective method based on spectrophotometric determination analysis providing satisfactory selectivity for routine analysis.

## **2. Material and methods**

### **2.1 Reagents and apparatus**

Niclosamide was purchased from Rock pharmaceutical Laboratories Pvt. Ltd. KPK Pakistan. Sodium hydroxide was purchased from DAEJUNG chemicals and metals Co, Ltd Korea. Tetrahydrofuran was purchased from RCI Labscan Limited, Thailand. Phenol and Cholinechloride were purchased from Sigma-Aldrich, Germany. Ethylene glycol, Glycerin and Malonic acid were purchased from DAEJUNG chemicals and metal Co, Ltd, Korea.

Niclosamide solution was prepared by dissolving 24 mg in 50 mL of 0.1M Sodium hydroxide solution. A 1 mL of this solution was further diluted up to 5 mL with methanol. A spectrophotometer (Shimadzu UV-Modal) with 1.0cm Galax cells was used for absorbance measurements. Ultra Sonic bath model Power sonic 603 (frequency 40 KHz, power 350 W) was used for sample preparation. Centrifuge (Volteac) was used to speed-up the phase separation.

### **2.2 Procedure**

#### **2.2.1 Synthesis of DES**

A deep eutectic solvent is always a mixture of two or three components that are able to interact with each other by hydrogen bonds. Choline chloride (ChCl), as quaternary ammonium salt, was used as a hydrogen bond acceptor (HBA) while other compounds (Urea, Citric acid, Ethylene glycol and Phenol) were used as hydrogen bond donors (HBD). Deep eutectic solvents, made of ChCl + Urea, ChCl + Phenol, ChCl + Ethylene glycol and ChCl + Citric acid, were prepared in a definite molar ratio by mixing weighted components in a vial with magnetic stirrer. ChCl +



Phenol was stirred at 298 K, ChCl + Urea at 298 K, ChCl + Ethylene glycol at 373 K and ChCl + Citric acid at 342 K. Preliminary studies revealed that DES based on ChCl and Phenol with a molar ratio 1:2 was the most effective one for Niclosamide extraction. Thus, this system was further investigated in upcoming steps.

### 2.2.2 Buffer system

To optimize the pH during extraction, the analytical signal was measured after extraction in presence of six different buffers at pH 2, 4, 6, 8, 10 and 12. The buffer, having pH 2, was prepared by mixing H<sub>3</sub>PO<sub>3</sub> (85%) and 3.118 g of NaHPO<sub>4</sub> in 100 mL of deionized water. A pH 4 buffer was prepared by dissolving 5.76 mL CH<sub>3</sub>COOH and 1.54 g CH<sub>3</sub>COONa in 100 mL of deionized water. A pH 6 buffer was prepared by dissolving 0.5 mL acetic acid (99%) and 11.7 g CH<sub>3</sub>COONH<sub>4</sub> in 100 mL of deionized water. A pH 8 buffer was prepared by dissolving 0.8 mL NH<sub>4</sub>OH and 10.7 g NH<sub>4</sub>Cl in 100 mL of deionized water. A pH 10 buffer was prepared by dissolving 4.5 g NH<sub>4</sub>Cl in 20 mL deionized water and 35 mL of 10 M NH<sub>3</sub> in 30 mL of deionized water and diluted up to 100 mL. A 2 mL of the buffer were added to each sample tube to adjust the pH.

### 2.2.3 Extraction and determination of Niclosamide

A 1 mL of Niclosamide standard stock solution (prepared with a buffer of pH 10) was taken into a graduated tube. The sample was diluted with methanol up to 10 mL. Next, 0.5 mL of deep eutectic solvent (DES) was added. This mixture was mixed in a sonicator for 5 min at 60°C. Afterwards, 0.5 mL of Tetrahydrofuran (THF) was added to increase the separation of aqueous and non-aqueous phase. The sample was then stirred for 2 min, followed by a centrifugation for 3 min at 4000 rpm. The resulting upper layer was transferred to a vial and diluted up to 5 mL. Absorbance was measured through UV Visible spectrophotometer at 378 nm, while the remaining part was discarded.

The calibration curve was performed on the basis of 10 standard solutions of Niclosamide ranging from 4.8 to 48 µg/L. Each solution was analyzed in triplicate. The absorbance at optimized wavelength of 378 nm was plotted versus concentration of the standard. The linearity range of calibration curve was evaluated on the basis of coefficient of determination (R<sup>2</sup>).



### 3. Results and discussions

#### 3.1 Selection of deep eutectic solvent as extractant

During preliminary studies, several DESs were tested as possible extractants for Niclosamide, including ChCl + Urea, ChCl + Citric acid, ChCl + Ethylene glycol and ChCl + Phenol at different molar ratios. Interestingly, ChCl and Phenol (1:2) provided the maximum recovery and thus selected for further studies. Comparing the structures of HBD compounds used in this study, it is clear that only phenol can provide sufficient  $\pi$ - $\pi$  interactions with the target analyte, which increases the extraction effectiveness. The results of this study are illustrated in Figure 1.

Further studies were related to optimization of HBA to HBD molar ratio for ChCl -Phenol DES. The selection was based on the absorbance measurements performed for extracts obtained during series of sample preparation protocols for standard solutions (10mg/L). Firstly, on the basis of standard solution of Niclosamide, an optimal wavelength was selected as 378 nm (see Figure 2). Preliminary studies confirmed that none of the used DESs had a cut-off value reaching the optimal UV absorption wavelength of Niclosamide.

Also, different DESs were examined with ChCl and Phenol by varying the molar ratio, as follows 2:1, 1:1, 1:2 and 1:3. It was observed that the application of ChCl and Phenol at molar ratio 1:2 exhibited the maximum recovery of the analyte, and consequently selected for further studies.

#### 3.2 Selection of optimum wavelength with maximum absorption

The pre-concentrated Niclosamide solution was scanned for absorption measurements through UV-visible spectrophotometer ranged from 300 to 800 nm. DES diluted with methanol was used as blank for this measurement. On the basis of obtained spectrum, a maximum of absorbance was observed at 378 nm, which was selected as optimum wavelength for further studies. The results of these experiments are illustrated in Figure 2.

#### 3.3 Optimization of donor phase pH

It is well known that pH strongly affects extraction effectiveness, especially in case of biomolecules. To optimize the pH of donor phase during extraction, this parameter was investigated using six different buffers of pH 2, 4, 6, 8, 10 and 12. A 2 mL of buffer solution was added to each standard sample to adjust the pH. Absorbance was measured for extracts obtained



at different pH. There was a continuous increase of extraction effectiveness observed along with increase of pH. Niclosamide is a weak acid and its pKa value is approximately 5.6. At pH 7.4, almost 99% of the Niclosamide molecules exist in deprotonated form. The distribution coefficient of Niclosamide in water decreases with increase in pH (Specifications, 2002, Alemu et al., 2003). Thus, it is expected that increase of the pH of donor phase should result with increased recovery of this analyte. This part of the studies confirmed this hypothesis. The best performances were obtained for pH 8, as shown in Figure 3; and this pH was selected as the optimum pH for further experiments.

### **3.4 Optimization of DES volume**

Final optimization of extraction was performed in relation to the volume of DES, which varied from 100 to 800  $\mu\text{L}$  with fixed amounts of other reagents. The optimal value of this parameter depends on two contradictory effects. First one, the capacity of the extractant in relation to analyte, and second one, the dilution effect. Increased amount of extractant decreases the concentration of analyte in the extract. The studies revealed that a gradual increase in absorbance was observed with increase of DES volume till 300  $\mu\text{L}$ . After 300  $\mu\text{L}$ , no distinct change was observed; thus, this volume was selected as the optimum for further studies. The results of these experiments are illustrated in Figure 4.

Nowadays, rapid procedures are expected to provide a high throughput of analyzed samples. To speed up the phase separation process after extraction, a demulsifier agent was used. According to preliminary studies, THF addition was found be useful for this purpose. The addition of THF was optimized in respect to its volume in relation to two parameters – phase separation effectiveness and recovery of the analyte. To determine the optimum volume of THF addition, a different volume of THF was added into five different samples ranging from 100 to 800  $\mu\text{L}$  for 10 mL of bulk solution. The results of this experimentation are given in Figure 5. This part of the studies revealed, that THF volume of 0.3mL per 10mL of sample solution gives a maximum recovery as well as fast phase separation, thus it was selected as the optimum ratio for this method and used in further studies.

### **3.5 Validation of the analytical method**

The proposed method has been validated in terms of linearity, the limit of detection (LOD) and quantitation (LOQ), precision and recovery. The data obtained from the validation process



fulfills the acceptance criteria. Thereby, it is concluded that the method is reliable and can be used for the assay of Niclosamide in routine practice. Below, detailed results regarding each aspect are discussed.

### 3.5.1 Limit of detection (LOD) and quantitation (LOQ), linearity

The calibration curve indicated statistical results of the determination coefficient ( $R^2$ ) more than 0.995. The results shown in the following tables and calibration curve indicate (see Figure 6) that the absorbance of sample is directly proportional to the concentration of the analyte, following the Beer-Lambert law. The limit of detection (LOD) was calculated using equation (1), as follows:

$$\text{LOD}=(3.3*S_a)/b \quad (1)$$

where  $S_a$  is the standard deviation of the intercept of calibration curve, while  $b$  is the slope of the calibration curve. The limit of quantitation (LOQ) was calculated using equation (2), as follows:

$$\text{LOQ}=(10*S_a)/b \quad (2)$$

LOD and LOQ values were found 0.112 and 0.374 $\mu\text{g/L}$ , respectively. The linearity was confirmed for concentration between 4.8-48  $\mu\text{g/L}$ . These results are illustrated in Figure 6.

### 3.5.2 Application of developed method for real samples and recovery

The analytical performance of the developed method allows its application in the environmental monitoring of water samples as well as process control of industrial effluents in the pharmaceutical industry. Niclosamide samples in form of tablets were collected from different pharmaceutical industries. The first sample was Meson 500mg (Niclosamide) in bulk tablets of Shaigan Pharmaceutical Pvt Ltd Rawalpindi. The second sample (Yomezan) was collected from Rock Pharmaceutical industry KPK Pakistan consisting of 500 mg Niclosamide in a bulk mixture. These tablets were finely ground in a mill. A stock solution was prepared by dissolving 24 mg Niclosamide in 50 mL of 0.1M Sodium hydroxide aqueous solution. Further dilution was done through methanol to get desired concentration.

Sample preparation procedure was performed as described in section 2.2.3. Obtained extracts were analyzed by spectrophotometer. A scheme of developed procedure is graphically presented in Figure 7. The absorbance for these samples was measured and percent recovery with relative





standard deviation was calculated. The percent recovery was calculated using equation (3). The obtained values should range from 98 to 102%.

$$\% \text{ Recovery} = C_f / C_a \times 100\% \quad (3)$$

where  $C_f$  is analyte concentration found in spiked sample and  $C_a$  is analyte concentration added as spike.

The percent recovery in Yomezan and Meson samples was 97.82% and 98.10%, respectively, with  $RSD < 2\%$ . This method was found to be quite effective for both higher and lower concentrations. The theoretical value is within the 95% confidence limit. The results are enlisted in Table 1.

Regarding the precision of analytical procedure is within the closeness of results in a repeated set of experiments of the homogeneous sample. As shown in Table 2, two types of precision were determined during this study. For the inter-day precision, a homogeneous sample was analyzed by different analyst in different days to describe the reproducibility of the method; and intraday precision, also called repeatability, has been evaluated as well. In general, the method indicates intraday precision/reproducibility over a short period of time at the same operating condition. It was performed at a very short interval of time within the same laboratory, same analyst and same equipment. Importantly, the results presented acceptable RSD values that meet the criteria of less than 2%.

### 3.6 Comparison of the proposed method and other reported methods

This method was compared with already existing methods for Niclosamide analysis. It is worth mentioning that this study introduces, for the first time, an extraction procedure for Niclosamide based on DESs. A comparison of the developed method with other methods is presented in Table 3. Specific analytical parameters, such as LOD, linearity range, detection method and used reagents, were compared. As mentioned previously, these reported methods are based on RP-HPLC, HP-TLC (Paghadar and Vadia, 2019), nanohybrid sensor and voltammetric detection (Zhang et al., 2017), indirect spectrophotometry (S Othman and H Sultan, 2018), as well as electrochemistry (Dede et al., 2014).

In principle, electrochemical methods involve the reduction of nitro group in Niclosamide structure (Alemu et al., 2003, Abreu et al., 2002). Nevertheless, Niclosamide reduction process

occurs with low sensitivity and reproducibility, in which the surface fouling causes a high overpotential at the unmodified electrodes, and in the case of polarography, a very narrow linear range limits the application of this method (Ghalkhani and Shahrokhian, 2010). Also, nanomaterial-based methods are comparatively more expensive (Zhang et al., 2017). On the other hand, RP-HPLC methods commonly use large amounts of organic solvents and thus generate high quantities of waste. This exponentially leads to some issues in terms of ecological impact and operator safety. For instance, acetonitrile is a flammable, volatile, and toxic solvent, which has a negative impact on the environment along with health safety issues (Guideline, 2005, Sheldon, 2012).

In above reported methods, LC-MS/MS using Multiple Reaction Monitoring (MRM) offers a highly selective and sensitive quantification (Doran and Stevens, 2014); however, when compared with our new method, both are characterized by comparable LOD, but LC-MS/MS represents higher costs and complexity of instrumentation. At this point, we recognize that the main limitation of our developed method deals with the multi stage procedure demanding some experience in laboratory practice. Secondly, concentration of reagents should be manually adjusted that is time consuming. Finally, full automation of the procedure would require a custom-design sample-prep instrument.

#### **4. Conclusions**

In this study, a novel and “green” DES-LPME method was developed for the pre-concentration and spectrophotometric determination of Niclosamide. This is a green chemistry approach where deep eutectic solvents were used. In fact, this is the first-ever method developed for Niclosamide extraction using green solvents. A standard protocol was developed for Niclosamide pre-concentration. All the necessary conditions of sample preparation and determination were optimized. The analytical parameters of this method were compared with documented methods for Niclosamide analysis, and it was found that this method has low detection limit, low RSD value and high recovery rate. LOD and LOQ were found to be around 0.112 and 0.374 $\mu\text{g/L}$ , respectively, which proves that the method based on spectrophotometric determination is highly sensitive. The reproducibility and repeatability for this new method were found <2%, pointing out its reliability for analytical purposes.



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## Conflict of interest

The authors declare no conflict of interest.

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## Figures

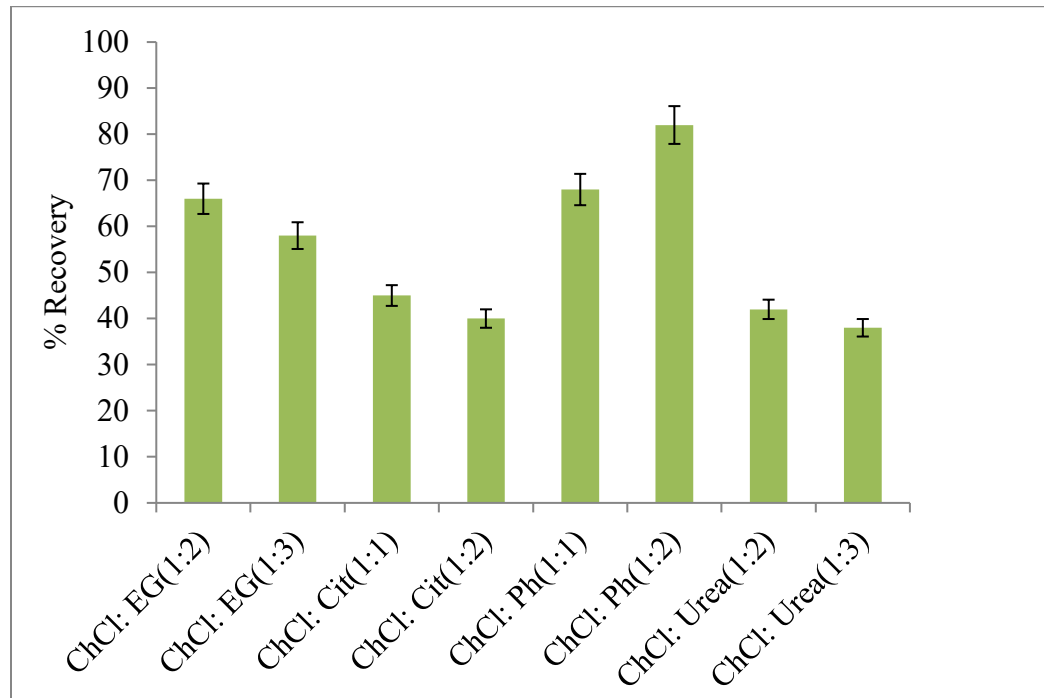


Figure 1: Preliminar evaluation and selection of DES. ChCl: Choline chloride, EG: Ethylene glycol, Cit: Citric acid, Ph: Phenol .

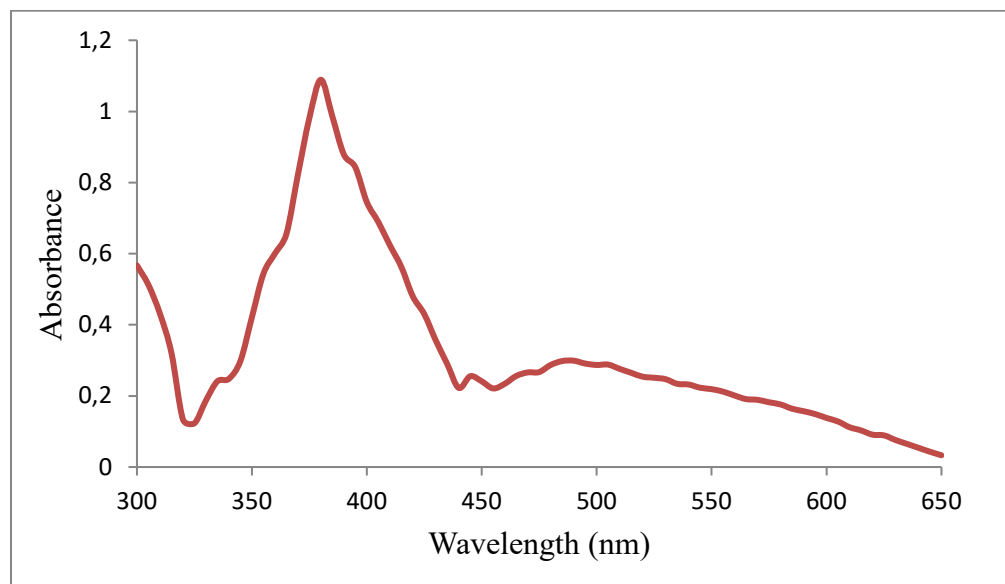


Figure 2: UV-Visible spectrum of Niclosamide.



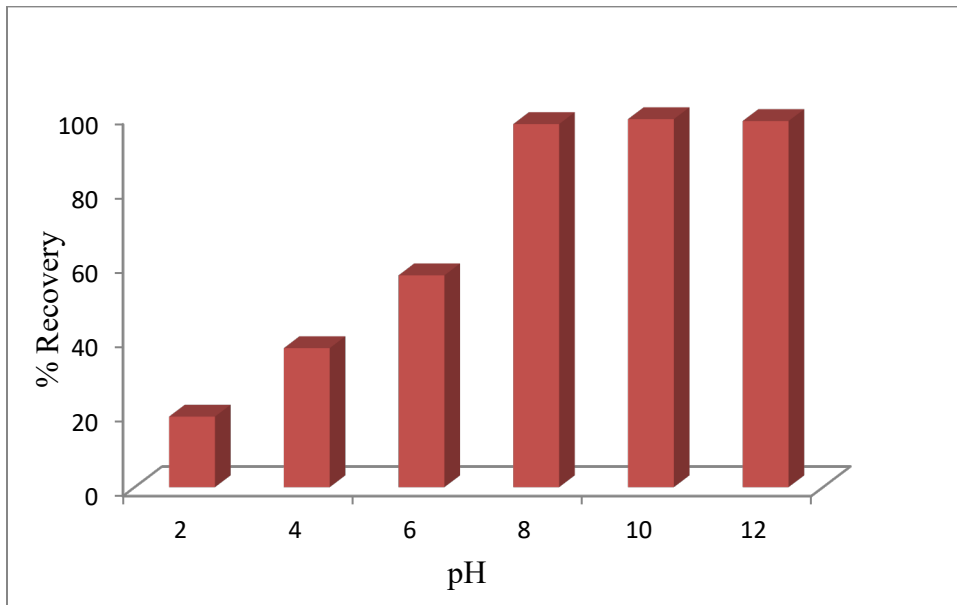


Figure 3: Recovery percentages (%) as function of pH.

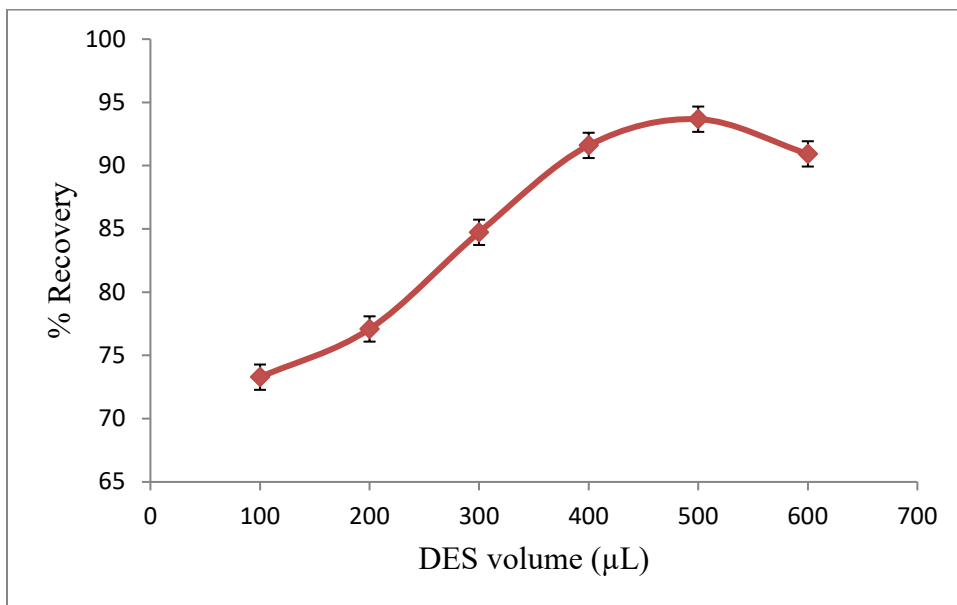


Figure 4: Optimization of DES volume.

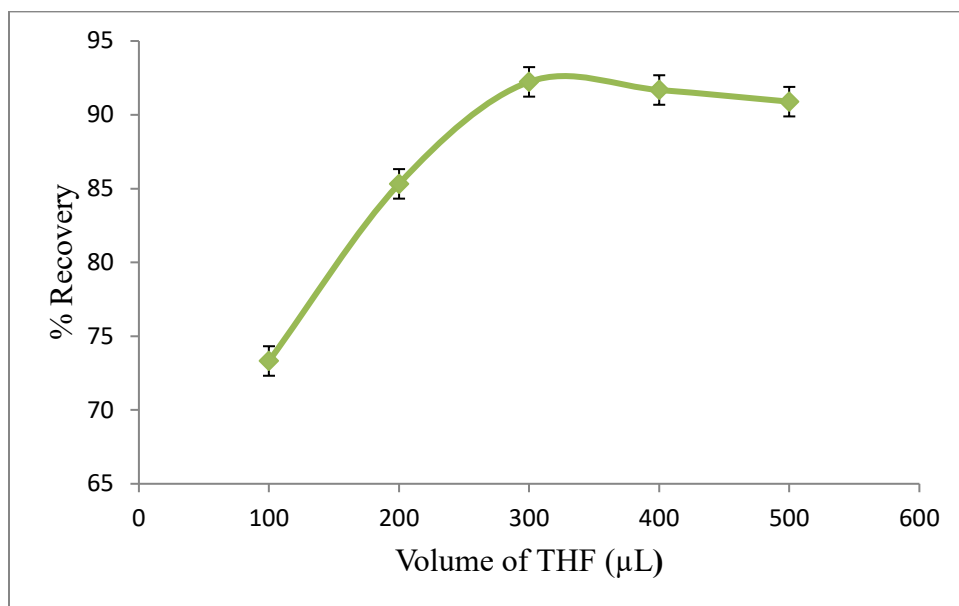


Figure 5: Optimization of Tetrahydrofuran (THF) volume

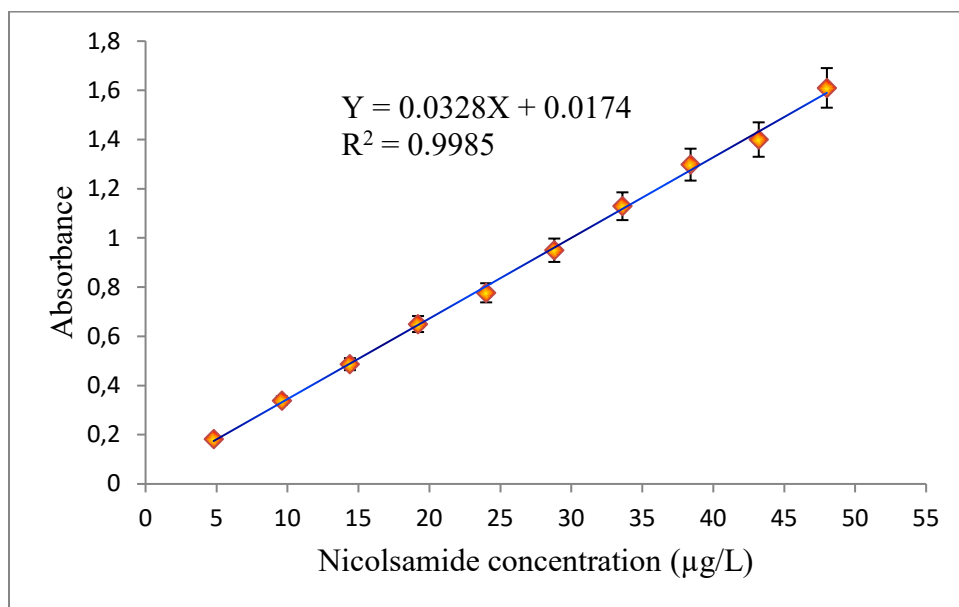


Figure 6: Calibration curve of Niclosamide.





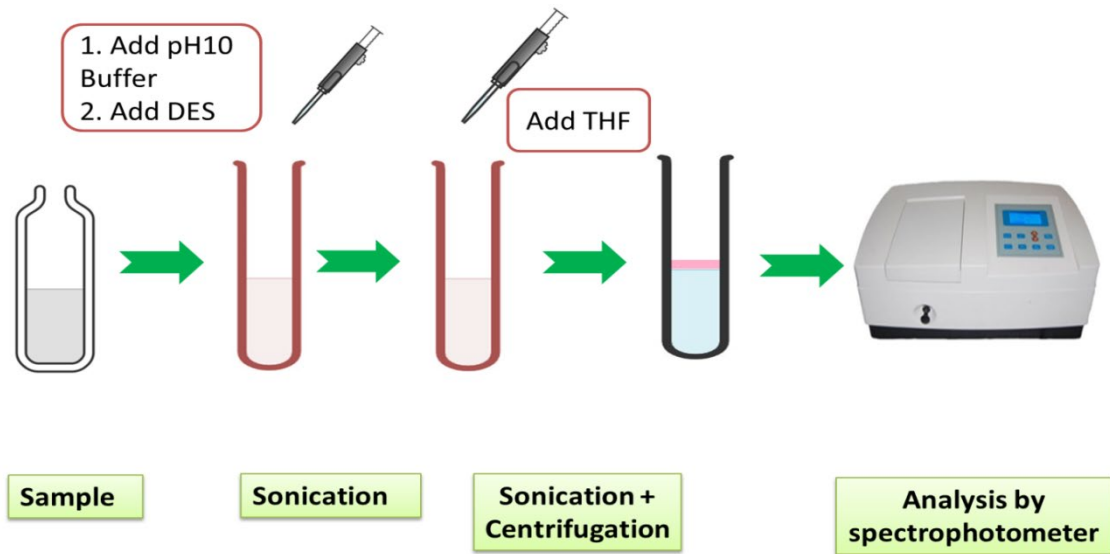


Figure 7: Graphical drawing of the proposed methodologies for sample analysis.

Table 1: Method validation and application on real samples

Tablet Sample	Amount taken (µg/L)	Amount measured (µg/L)	% Recovery
Yomezan	24	23.47	97.82
Meson	24	23.54	98.10
Water sample	Niclosamide added (µg/L)	Niclosamide found (µg/L)	% Recovery
Canal water	-----	18 ± 0.004	
	10	26.5 ± 0.006	94.64
	20	36.2 ± 0.002	95.26
	30	47.1 ± 0.004	98.12

Table 2 (a): Precision of method carried out in different days.

Day	Sample	Concentration (mg/L)	Absorbance	Average	RSD
Day 1	Niclosamide	0.019	0.737	0.736	0.150%
Day 1	Niclosamide	0.019	0.737		
Day 1	Niclosamide	0.019	0.735		
Day 2	Niclosamide	0.019	0.735	0.734	0.079%
Day 2	Niclosamide	0.019	0.734		
Day 2	Niclosamide	0.019	0.734		
Day 3	Niclosamide	0.019	0.733	0.733	0.079%
Day 3	Niclosamide	0.019	0.733		
Day 3	Niclosamide	0.019	0.732		

Table 2 (b): Method precision by different analyst

Analyst	Niclosamide concentration (mg/tab)
Analyst 1	499.21
Analyst 2	499.33
Analyst 3	499.01
Calculations	
Mean	499.18
Standard deviation.	0.1615
RSD%	0.0323

Table 3: Comparative study of present method with other reported methods

Method	Reagents	LOD ( $\mu\text{g/L}$ )	Linearity ( $\mu\text{g/L}$ )	Real samples	References
HPLC, Voltammetry detection	Palygorskitenano rods, Graphensnanosheets, $\text{LiClO}_4$ , $(\text{K}_3[\text{Fe}(\text{CN})_6])$ , Nicotinic acid, $\text{NiCl}_2$ , $\text{Na}_2\text{HPO}_4$	15	7.5 - 32	Paddy water	(Zhang et al., 2017)
RP-HPLC and HPTLC	Methanol, Ammonium phosphate, Silica gel G60 F254,	48	10 - $10 \times 10^3$	Tablets	(Paghadar and Vadia, 2015)
RP-HPLC, Spectrophotometric	Acetonitrile, Potassium dihydrogen phosphate, MethanolicHCl,	1000	$80 \times 10^3$ - $130 \times 10^3$	Pharmaceutical dosage	(Shah et al., 2016)
Electrochemical	Glassy carbon, $\text{K}_3[\text{Fe}(\text{CN})_6]$ , KCl, 2-Chloro-4-nitroaniline, Salicylic acid, Sodium perchlorate, $\text{NH}_3$ , NaOH	26	19.6 - 196	Tablets	(Alemu et al., 2003)
HPLC, UV detector	Ethyl acetate, Methylene chloride, Methyl iodide, Methanol, DMSO, Hexane, $\text{Na}_2\text{SO}_4$	600	----	River water and sediments	(Muir and Grift, 1980)
LC-MS/MS	5-Chlorosalicylic, Methanol, Acetonitrile	0.1	1 - 100	Water	(Doran and Stevens, 2014)
DES extraction, Spectrophotometric detection	DES, THF, Methanol	0.112	4.8 - 48	River water, Tablets	<i>Present method</i>