JOURNAL OF APPLIED BIOANALYSIS, August. 2024, p. 62-72.
http://.doi.org/ 10.53555/jab.v10i2.155 (ISSN 2405-710X)
Vol. 10, No. 2

OPEN ACCESS

RESEARCH ARTICLE

# Pharmacokinetics of Niclosamide in Rat Plasma by RP HPLC via Intranasal and Intraperitoneal Route of Administration

Nilesh Mahajan[1], Suchitra Mishra[2*], Amol Warokar[3], Sachin More[4] , Anil Pethe[5]

[1, 2,3,4]*Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, Maharashtra, India.*
[5]*Datta Meghe College of Pharmacy, Wardha, Maharashtra, India.*

**Background: Niclosamide (NIC) has been proposed as an interesting molecule for repurposing for antiviral activity. This study examined the comparative pharmacokinetic study of niclosamide by intranasal and intraperitoneal route in male Sprague-Dawley rats.**
**Material & Methods: The bioanalytical method was developed on Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for estimation of niclosamide in the presence of ibuprofen as an internal standard. An isocratic elution of mobile phase of 20 mM phosphate buffer; pH 4.5: methanol (15: 85 % v/v) was maintained at flow rate of 1.1 mL/min and effluent was monitored by Photo Diode Array (PDA) detector at 254 nm.**
**Results: After a single 20 mg/kg intraperitoneal dose, the maximum concentration ($C_{max}$) of niclosamide was found to be 9031±0.003 ng /ml, maximum time to reach peack concentration ($t_{max}$) was 2 hr and half-life ($t_{1/2}$) of was found to be 0.55845±0.001 hours, whereas after intranasal administration the $C_{max}$ of niclosamide was found to be 6109±0.0026 ng /ml, $t_{max}$ was 5 hr and half-life ($t_{1/2}$) was found to be 1.617186±0.0017 hr. The plasma peak concentration of niclosamide after two hours was 6.8 µg after 2 hr of intranasal administration and gradually decrease with time, whereas there were no significant concentration of NIC detected in lungs by intraperitoneal administration. Two-fold increase in area under curve (AUCO-t) and Mean residence time (MRT) with diminished clearance after administration of niclosamide via intranasal route in lungs.**
**Conclusion: Relatively higher concentration of niclosamide was estimated in lungs via intranasal while in plasma via intraperitoneal route of administration in rats. It is imperative to elucidate the pharmacokinetic characteristics of niclosamide in human subjects prior to its prospective application in individuals afflicted with SARS-CoV.**

**Keywords:** Pharmacokinetics, Drug repurposing, Niclosamide, RP-HPLC, Intranasal Route of Administration, Mean residence time

## INTRODUCTION

Niclosamide (NIC), chemically denoted as 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (refer to Figure 1), is recognized by the Food and Drug Administration (FDA) as an anthelmintic medication and is classified as a critical medicine by the World Health Organization (WHO).[1] NIC has been used for the treatment of tapeworm infection for many decades.[2] NIC has been proven to be a powerful drug preference for repurposing in the treatment of SARS-CoV-2 with the aid of *in vitro* research with Vero E6 cells.[3] As of this moment, there exists no empirical evidence evaluating the pharmacokinetics of NIC following any mode of administration in a zoological model aimed at the mitigation of SARS-CoV-2. Nonetheless, there are currently 10 articles listed on clinical trials investigating both oral and inhalational formulations of NIC available at www. clinicaltrials.org.

The literature review has highlighted a variety of analytical methods for the quantitative analysis of NIC in both bulk forms and complex preparations.[4,5,6] Research has uncovered numerous approaches for measuring the concentration of NIC, whether it is being analyzed by itself or in conjunction with other active pharmaceutical ingredients (APIs). The measurement of NIC in combination with thiabendazole[7] and drotaverine hydrochloride[8] using spectrophotometry, as well as

***Corresponding Author:** *Dr. Suchitra S. Mishra*
**Department of Pharmaceutics, Dadasaheb Balpande College of Pharmacy,Besa, Nagpur. 440037, Mobile No: +91-9146945512, Email: suchitramishra36@yahoo.com*

spectrofluorometry,[9] has been documented. A range of chromatographic techniques have also been proposed, including high-performance liquid chromatography (HPLC)[10,11] and gas chromatography (GC).[12,13] Additionally, electrochemical methods for NIC quantification have been described, utilizing square-wave voltammetry[14] and cyclic voltammetry at a glassy carbon electrode.[15]

As in step with the pronounced literature, no reproducible RP HPLC method was found for quantification of NIC, so it has evoke into an idea to expand HPLC technique for quantitative analysis and bioanalytical method of NIC from bulk. In case of Covid, pneumonia, and lung fibrosis infection, maximum concentration of drug need to be available at targeted site i.e. lungs. Therefore it is imperative to develop a product to be administered by inhalation for targeted drug delivery and to determine pharmacokinetic and tissue distribution of NIC and validate a quantification approach of NIC in biological matrices. To accurately and efficiently measure NIC levels, a rapid and precise bioanalytical approach utilizing RP-HPLC was established for the effective quantification of NIC.[16] Once the method had been developed and verified as consistent with the In accordance with industrial guidelines for bioanalytical method.

It was used for the estimation of NIC in different biological samples and compared the pharmacokinetic data obtained between the intraperitoneal and intranasal routes of administration.

## MATERIALS

High-quality Niclosamide (NIC) of pharmaceutical ingredient grade was sourced from Sigma Aldrich, while Ibuprofen (IBP) was kindly provided as a processed sample by Zim Laboratory Ltd., located in Nagpur, India. Methanol and water conforming to HPLC specifications were procured from Merck Chemicals, located in India. Additionally, analytical-grade chemicals such as ammonia, orthophosphoric acid, dimethyl sulfoxide (DMSO) and potassium dihydrogen phosphate were secured from Loba Chemie Pvt. Ltd., based in Mumbai, India. Prior to conducting the RP-HPLC for the purpose of detecting NIC, the mobile phase was subjected to filtration using a membrane filter with a pore size of 0.2 µm, which was obtained from Millipore, Bedford, MA, USA.

### Instruments

The analysis via chromatography was conducted using a binary gradient RP-HPLC: (LC-20 AD Shimadzu, Japan), equipped with a dual pump. Data processing was performed using the HPLC Lab solution software.

Ultrasonic bath: Provided by Bio Technique India.
Refrigerated centrifuge: The REMI model CM 12 Plus, based in India.

### Animals

In the context of bioanalytical, pharmacokinetic, and tissue distribution studies, male Wistar rats of sound health were employed. These rodents, with an average mass of 200±50 grams, were maintained in an environment regulated for temperature and humidity, specifically at 22±2 °C and 60% relative humidity, respectively. In the week leading up to the study, the rats were given standard lab chow and water ad libitum. They were fasted overnight just before the commencement of the experimental procedures.

### Development of the Bioanalytical Method

### HPLC Equipment and Chromatographic Setup

The analytical procedure was established utilizing a Shimadzu HPLC-2010 system, which includes a photodiode array detector. The stationary phase utilized was a Shimpack C18 column (dimensions: 250 × 4.6 mm, particle size: 5 µm). The composition of the mobile phase consisted of methanol and potassium dihydrogen phosphate buffer in a volumetric ratio of 85:15, with the flow rate precisely regulated at 1.1 ml/min. Prior to its application, the mobile phase was filtered through a 0.2 µm membrane filter. Analyte detection during the elution process was performed at a wavelength of 220 nm, employing an injection volume of 20 µl. Modifications to the mobile phase, including adjustments to its composition, pH, and flow rate, were explored in various chromatographic experiments to optimize peak resolution.[17,18] The internal standard (IS) and NIC exhibited retention times of 6 min and 11 min, respectively. The peak area for 5µg of NIC was recorded at 390,797, with peak heights for IS and NIC observed at 2,091 mAU and 30,772 mAU, respectively. The tailing factors for the internal standard (IS) and niclosamide (NIC) were determined to be 0.98 and 1.018, respectively. Moreover, the number of theoretical plates for IS and NIC was calculated to be 15,308 and 16,478, respectively. Furthermore, the Height Equivalent to a Theoretical Plate (HETP) for IS and NIC was measured at 9.799 and 9.103, according to USP standards.

### Standard Solution Preparation

A precise quantity of 20 mg of NIC was weighed and introduced into a 25 mL volumetric flask, followed by dissolution in methanol up to the mark of the flask. The solution underwent ultrasonication for a duration of 10 min. A subsequent step involved the transfer of a 2.5 mL portion of this primary solution into a 10 mL volumetric flask,

which was then filled to the marked volume with methanol, resulting in a series of NIC working solutions of varying concentrations, incorporating an internal standard. These prepared solutions, encompassing both stock and working variants, were preserved at 4 °C in conditions devoid of light exposure and were brought to room temperature before usage.

**Technique for Sample Extraction**

Blood specimens were acquired via the retro-orbital plexus of the rats and deposited into tubes designed to inhibit coagulation (sourced from Himedia Laboratories, Mumbai, India). Following collection, the blood samples were subjected to centrifugation at 12,000 rpm for a span of 10 min at a temperature of 4°C, after which the plasma, forming the supernatant layer, was meticulously extracted.[19,20] For drug detection in lung tissue, the tissue was excised, washed with 0.9% saline to remove any adhering blood and debris, and then blotted dry. Each organ was weighed accurately and then homogenized in methanol, using three times the organ's weight, to produce a methanol homogenate. These homogenized samples, whether plasma or tissue, were then stored at 2 °C in sealed vials until further analysis, which involved a straightforward protein precipitation process.[21]

**Single-step protein precipitation**

Protein precipitation was achieved through a straightforward method wherein 100 µl of rat plasma or tissue homogenate was combined with 1 mL of methanol in a 2 ml Eppendorf tube. The solution was subjected to vigorous agitation for 5 min, followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The clarified supernatant obtained post-centrifugation was then evaporated under nitrogen gas. The dried residue was reconstituted in 1 ml of methanol, supplemented with an internal standard, filtered through a 0.2 µm syringe filter, and a 20 µl sample of this preparation was subjected to analysis via high-performance liquid chromatography.[22,23]

**Bioanalytical technique validation**

Validation of the bioanalytical method was conducted to confirm its reliability and consistency, in compliance with the Bioanalytical Method Validation Guidance provided by the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM) in May 2018. The validation process encompassed a comprehensive evaluation of essential parameters including system suitability, specificity, matrix effects, recovery efficiency, linearity, precision, accuracy, robustness of the method, and stability of the analytes within the biological matrix.[24]

**Precision and accuracy**

The precision and accuracy of the analytical methodology were determined by conducting intra- and inter-day evaluations at three distinct concentration levels: low quality control (LQC) at 2.5 ng, medium quality control (MQC) at 2500 ng, and high quality control (HQC) at 4000 ng. The sample was run in three replicates for intraday precision and . Same concentrations were used for interday precision and accuracy for three cosecutive days .[25,26]

**Linearity assessment**

A calibration curve was established by plotting the ratio of NIC's area under the curve to that of the internal standard, Ibuprofen, against NIC's concentration levels. The regression equations were derived using the partial least squares regression method. Linearity was assessed over NIC concentration ranges of 2.5, 5, 10, 50, 100, 500, 1000, and 5000 ng/mL in specific tissue samples, with Ibuprofen (IBP) serving as the internal standard at a concentration of 1 µg/mL. The quantification of the analyte relative to the internal standard as a function of concentration was determined through a weighted ($1/x^2$) least squares regression analysis.[27,28] The coefficient of determination ($r^2$) served as an indicator of the method's analytical efficacy, with a resultant value of 0.9988. The percent relative standard deviation (%RSD) for each designated concentration level was anticipated to be confined within ±15%, with the exception of the lower limit of quantification (LLOQ), for which a permissible deviation of ±20% from the nominal concentration was considered acceptable.[29]

**Quantification Limits**

The establishment of the lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) was predicated on identifying the minimal concentration at which the observed peak height of the drug exceeded the baseline noise by factors of at least three and ten, respectively.[30] Assessment of matrix effects and recovery efficiencies was conducted at three distinct concentration levels—low, medium, and high as shown in table 2. The effectiveness of the extraction methodology was verified through the comparison of peak area ratios obtained from the biological matrix, enriched with quality control (QC) samples, before and subsequent to centrifugation processes.[30,31]

**Robustness**

The stability of the HPLC methodology was evaluated by altering variables such as the

composition of the mobile phase by ±2%, the pH of the mobile phase by ±1, the flow rate by ±0.2 mL/min, and the detection wavelength by ±2 nm. These modifications were made to assess the method's reliability in producing consistent results under slightly varied conditions.[32]

**Specificity**

To assess the specificity of the developed method, chromatograms of a blank matrix were generated and compared with those obtained from samples containing NIC. This comparison aimed to ensure that the method could accurately identify and measure NIC without interference from other substances.[33]

**System suitability test**

Analytical device overall performance was evaluated earlier and all through the evaluation by means of system suitability tests, which were done to evaluate the behavior of the chromatographic machine along with the theoretical plate range (N) and tailing factor (T). (Table 1)

**Sample stability**

The stability of samples was rigorously assessed by analyzing three replicates at two analytical concentration levels (low and high) across three distinct conditions: retention at ambient temperature for a duration of 24 hours, exposure to three freeze-thaw cycles, and maintenance at 4 °C for a period of 10 days (refer to Table 3). This evaluation was performed to ascertain the preservation of sample integrity over time and through varied storage scenarios.[34]

**Pharmacokinetic Study**

The pharmacokinetic assessment involved measuring NIC concentrations in both tissues and plasma. Twelve Wistar rats were utilized for this purpose, divided into two groups of six each.[34,35] One group received NIC intraperitoneally, while the other was administered NIC intranasally, with all administrations conducted on an empty stomach. The study protocol received approval from the Institutional Animal Ethics Committee [Approval No.DBCO/IAEC/1426/20-21/P1], adhering to CPCSEA guidelines. The formulation for intraperitoneal injection consisted of 10% dimethyl sulfoxide, 30% polyethylene glycol, 20% 0.05 N sodium hydroxide solution, and 40% saline. Blood specimens (0.5 mL) were procured at time intervals of 0.5, 1, 2, 3, 4, and 6 hours following administration from the retro-orbital plexus utilizing heparinized capillary tubes under ether anesthesia. Subsequent to collection, plasma was isolated via centrifugation and preserved at −20 °C for further examination.[36,37]

The plasma specimens were subjected to a singular protein precipitation technique, reconstituted in 1 mL of methanol, filtered through a syringe filter, and subjected to HPLC analysis at 220 nm. Additionally, six rats from the second group were euthanized using a ketamine and xylazine overdose at 2, 4, and 6 hrs post-dose. Lung tissue samples were similarly prepared, involving homogenization and processing through a one-step protein precipitation method, followed by liquid-liquid extraction.[38,39] These samples were then prepared in 1 mL of methanol with the internal standard, filtered, and subjected to HPLC analysis at 220 nm.

**Statistical analysis**

Pharmacokinetic parameters were calculated using PK Solver software, employing a non-compartmental analysis approach. Key parameters evaluated included the peak plasma concentration ($C_{max}$), time to achieve peak concentration ($t_{max}$), plasma concentration-time curve (AUC), mean residence time (MRT), half-life ($t_{1/2}$), and total area under the curve (AUC) of NIC.

## RESULTS

**Development of the Bioanalytical Technique**

The bioanalytical method was developed for the precise quantification of NIC concentrations in rat plasma and tissue specimens. The optimized mobile phase comprised potassium phosphate buffer (pH 3.5) and methanol, utilizing an isocratic elution profile at a ratio of 15:85 and a flow rate of 1.1 ml/min. The elution times recorded for NIC and the internal standard were 11.18±0.0025 minutes and 5.99±0.023 minutes, respectively, as outlined in Table 1 and figure 1. Detection of these compounds was performed at a wavelength of 220 nm using a photodiode array (PDA) detector.

**Assessment and Validation of the Assay**

Before conducting each analysis, the system's suitability was assessed through parameters like tailing factor, number of theoretical plates, height equivalent to a theoretical plate (HETP), and retention time, ensuring compliance with ICH guidelines, as presented in Table 1.

The method's efficacy in NIC quantification without any interfering matrix was confirmed by evaluating peak purity and standard curve linearity across a range of 2.5– 5000 ng/mL (figure 2) for both plasma and tissue samples. The calibration curve for NIC, plotted with these concentrations, is illustrated in figure 3.

NIC's extraction recovery and the effect of the tissue matrix at concentrations of 50, 500, and 5000 ng/mL were observed to be between 91.12– 93.55%, 93.55– 105.43%, and

97.84– 103.56%, respectively, indicating negligible matrix interference in NIC quantification (Table 3).
The method's robustness was assessed by minor adjustments to the mobile phase composition, with observed variations in chromatographic parameters staying below 2% relative standard deviation (Table 3).
Stability tests compared chromatograms from stored solutions against those freshly prepared, over periods up to 10 days at 4 °C, through four freeze-thaw cycles, and after 24 hr at room temperature for both plasma and tissue samples. The variance between stored (SS) and fresh (FS) samples was found to be under 5% (Table 3).

**Pharmacokinetic analysis**

A comparative pharmacokinetic comparison was conducted between NIC administered intraperitoneally and intranasally, based on established pharmacokinetic metrics such as area under the curve (AUC), time to reach peak concentration ($t_{max}$), mean residence time (MRT), peak concentration ($C_{max}$), half-life ($t_{1/2}$), area under the first moment curve (AUMC), steady-state volume of distribution (Vss), and clearance (CL), which are detailed in Table 4. The variation in plasma NIC levels over time following both intraperitoneal and intranasal administration is depicted in figures 4, 5, and 6.
Post intraperitoneal injection, NIC levels were traceable up to 8 hours, with the plasma concentration curve revealing a $t_{max}$ at 2 hr, a $C_{max}$ of 9031 ng/ml, and a $t_{1/2}$ of 0.55845 hrs. Conversely, NIC administered intranasally showed a $t_{max}$ at 4 hr, a $C_{max}$ of 6109 ng/ml, and a $t_{1/2}$ of 1.617186 hr, highlighting notable differences in $C_{max}$ and $t_{max}$ between the two administration routes. Intranasal administration demonstrated a near doubling in AUCO-t and MRT values alongside reduced clearance, as outlined in Table 4, indicating a significant decrease in NIC relative bioavailability via the nasal route due to its accumulation in the lungs. This suggests that nasal delivery could be a viable method for targeting lung localization, particularly for treatments targeting COVID infections.
Further examination of lung tissues from rats after intranasal NIC dosing revealed the presence of 6.8 µg of NIC in the lungs 2 hr post-administration, as recorded in Table 5, underscoring the drug's pulmonary deposition.

## DISCUSSION

A bioanalytical technique was developed to evaluate the comparative bioavailability of NIC through intraperitoneal and nasal administration. The chromatographic analysis ensured that the internal standard (IS) and NIC were distinctly separated without overlap from any matrix components. The calibration curve demonstrated a consistent linear relationship for NIC concentrations ranging from 2.5 to 5 µg/ml in both plasma and tissue samples. This method proved to be highly effective in detecting NIC concentrations, making it suitable for application in biological matrices. The drug accumulation records of lungs tissue homogenates with the aid of the nasal route in Table 5 shown a relatively higher quantity of drug deposited in the lungs, which showed that during cases of COVID and other lung infections, nasal direction management of NIC can be useful for targeted drug delivery.

## CONCLUSION

A suitable and robust bioanalytical technique has been developed to evaluate the comparative bioavailability of NIC through intraperitoneal and nasal administration. From the above results it has been concluded that nasal route is more efficient route of administration as compare to intraperitoneal route of drug administration in case of lungs infection treatment . In treatment of lungs infection in pneumonia and COVID condition, the nasal route can be used for localization of drug in lungs.

### *Executive summary*

- A bioanalytical method for NIC has been developed.
- Method validation was done at different levels, like linearity, robustness, system suitability, matrix effect, and stability.
- Drugs are administered by two different routes: intraperitoneal and intranasal.
- A comparative pharmacokinetic study has been done by both the route
- Accumulation of drug in lungs was determined by intranasal
- A significant amount of NIC found in the lungs indicated that the nasal route of administration can be used for further studies in the treatment of the lungs.

### *Future perspective*

- The process of bioanalytical method development and validation can be used further for the quantitative determination of NIC in different biological samples.
- A comparative study of the intraperitoneal route and the nasal route of administration provides a path for studying different API used for lungs infection.
- As this study has shown the deposition of NIC in the lungs, this fact can be further used for preparing different formulations like DPI to

increase the concentration and effect of the drug in the lungs.

- Patenting can also be done for novel formulations of NIC in the form of nanosystems.

## ABBREVIATIONS

***HPLC*** ***High Performance Liquid Chromatography***
***IS*** ***Internal Standard***
***LOD*** ***Limit of Detection***
***RP*** ***Reverse Phase***
***QC*** ***Quality Control***
***LQC*** ***Lower Quality Control***
***MQC*** ***Medium Quality Control***
***HQC*** ***Higher Quality Control***
***RSD*** ***Relative Standard Deviation***
***LLOQ*** ***Lower Limit of Quantification***


## ACKNOWLEDGEMENT

*The authors thanks the Principal and management for their support during the project work.*


## AUTHOR CONTRIBUTION

Dr. Suchitra S. Mishra has completed experimental work and drafted the manuscript. Dr. Amol Warokar has supported HPLC method development and validation, and Mr. Sachin More has assisted in pharmacokinetic studies. Dr. Nilesh Mahajan gave efficient hints and experimental support.


## FUNDING

This research work has been funded by Department of Biotechnology (BIRAC).


## AVAILABILITY OF DATA AND MATERIALS

Data will be made available on request.

## DECLARATIONS

## ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

*Not Applicable*

## CONSENT FOR PUBLICATION

*Not Applicable*

## COMPATING INTERESTS

*The authors declare that they have no compating interest*

## REFERENCES


1. World health organization. World Health Organization model list of essential medicines: 21st list 2019. World Health Organization; 2019.
2. Pawlowski Z, Allan J, Sarti E. Control of Taenia solium taeniasis / cysticercosis: from research towards implementation. Int J Parasitol. 2005;35(11-12):1221-32. doi: 10.1016/j.ijpara.2005.07.015, PMID 16143335.
3. Arshad U, *et al.* Prioritisation of potential anti-SARS-CoV-2 drug repurposing opportunities based on ability to achieve adequate target site concentrations derived from their established human pharmacokinetics. medRxiv, 2020.
4. Van Tonder E, Devilliers M, Simple HJ. robust and accurate high-performance liquid chomatography method for the analysis of several anthelmintics in veterinary formulations. J Chomatogr. *A*. 1996;729:267-72.
5. Cholifah S, Kartinasari WF, Indrayanto G. Simultaneous HPLC determination of levamisole Hcl and anhydrous NIC in veterinary powders, and its validation. J Liq Chromatogr Relat Technol. 2007;31(2):281-91.
6. San-xia L, Bo J, Jun-wei W. HPLC method for content determination of compound NIC tablet. J Pharm Anal. 2013;33(2):317-21.
7. Onur F, Tekin N. Spectrophotometric determination of niclosamide and thiabendazole in tablets. Anal Lett. 1994;27(12):2291-301. doi: 10.1080/0003271 9408005983.
8. Daabees HG. Selective differential spectrophotometric methods for determination of niclosamide and drotaverine hydrochloride. Anal Lett. 2000;33(4):639-56. doi: 10.1080/00032710008543080.
9. Algarra M, Campos B, Rodriguez B, Jose E, Sanchez F. Niclosamide quantifification in methyl-β-cyclodextrin after derivatization to aminoniclosamide. J Inclus Phenom Macrocycl Chem. 2012;72(1):89-94.
10. Schreier TM, Dawson VK, Choi Y, Spanjers NJ, Boogaard MA. Determination of niclosamide residues in rain bow trout (Oncorhynchus mykiss and channel catfifish (Ictalurus punctatus) fillet tissue by high-performance liquid chromatography. J Agric Food Chem. 2000;48(6):2212-5. doi: 10.1021/jf990695r, PMID 10888524.
11. Caldow M, Sharman M, Kelly M, Day J, Hird S, Tarbin JA. Multi-residue determination of phenolic and salicylanilide anthelmintics and related compounds in bovine kidney by liquid chromatography– tandem mass spectrometry. J Chromatogr A. 2009;1216(46):8200-5. doi: 10.1016/j.chroma.2009.04.008, PMID 19426989.
12. Churchill FC, Ku DN. Extractive alkylation of 20, 5-dichloro 40 nitro salicylanilide (niclosamide) for gas– liquid chromatographic analysis. J Chromatogr. 1980;189(3):375-88.

doi: 10.1016/s0021-9673(00)80317-9, PMID 7380938.
13. John SJ, Geoffrey BP. Estimation of residues of the molluscicide, niclosamide, in bananas by gas–liquid chromatography of the heptaflfluorobutyryl derivative of the substituted aniline moiety. Pestic Sci. 1980;10(6):531-9.
14. Alemu H, Khoabane NM, Tseki PF. Electrochemical oxidation of niclosamide at a glassy carbon electrode and its determination by voltammetry. Bull Chem Soc Ethiop. 2003;17(1):95-106.
15. Abreu FC, Goulart MO, Brett AM. Detection of the damage caused to DNA by niclosamide using an electrochemical DNA-biosensor. Biosens Bioelectron. 2002;17(11-12):913-9. doi: 10.1016/s0956-5663(02)00082-9, PMID 12392939.
16. Ivan, S., Vesna, N., Ivana, S., Ljubisa, N., Mihajlo, S., 2013. Development and validation of a new RP-HPLC method for determination of quercetin in green tea. J. Anal. Chem. 68 (10), 906–911.
17. Purvin S, Vuddanda PR, Singh SK, Jain A, Singh S. Pharmacokinetic and tissue distribution study of solid lipid nanoparticles of zidovudine in rats. J Nanotechnol. 2014;2014:1-7. doi: 10.1155/2014/854018.
18. Bansal SK, Laylof T, Bush ED, Hamilton M, Hankinson EA, Landy JS, *et al.* Qualifcation of analytical instruments for use in the pharmaceutical industry: a scientific approach. AAPS PharmSciTech. 2004;5(1):1-8.
19. Xu S, Yu J, Zhan J, Yang L, Guo L, Xu Y. Pharmacokinetics, tissue distribution, and metabolism study of icariin in rat. BioMed Res Int. 2017;2017:4684962. doi: 10.1155/2017/4684962, PMID 29259982.
20. Saras-Nacenta M, López-Púa Y, Lípez-Cortés LF, Mallolas J, Gatell JM, Carné X. Determination of efavirenz in human plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl. 2001;763(1-2):53-9. doi: 10.1016/s0378-4347(01)00357-7, PMID 1171 0583.
21. Owens DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006;307(1):93-102. doi: 10.1016/j.ijpharm.2005.10.010, PMID 16303268.
22. Smith KM, Corporation W, Xu Y. Tissue sample preparation in bioanalytical assays. Bioanalysis. 2012;4(6):741-9. doi: 10.4155/bio.12.19, PMID 22452264.
23. Usami YU, Oki TO, Nakai MN, Sagisaka MS, Kaneda TK. A simple HPLC method for simultaneous determination of lopinavir, ritonavir and efavirenz. Chem Pharm Bull. 2003;51(6):715-8. doi: 10.1248/cpb.51.715.
24. Dolan JW. System suitability. LC Troubl. 2004;17(6):328-32.
25. Khan W, Sharma SS, Kumar N. Bioanalytical method development, pharmacokinetics, and toxicity studies of paromomycin and paromomycin loaded in albumin microspheres. Drug Test Anal. 2013;5(6):453-60. doi: 10.1002/dta.339, PMID 22447374.
26. Bhinge SD, Malipatil SM, Sonawane LV. Bioanalytical method development and validation for simultaneous estimation of cefxime and dicloxacillin by RP-HPLC in human plasma. Acta Chim Slov. 2014;61(3):580-6. PMID 25286213.
27. Jagadeeswaran M, Gopal N, Pavan K, Siva T. Quantitative estimation of lopinavir and ritonavir in tablets by RP-HPLC method. Pharm Anal Acta. 2012;3(5):3-5.
28. Kiran BV, Rao BS, Dubey SS. Development and validation of a reversed-phase HPLC method for the determination of efavirenz in pharmaceutical dosage forms by internal standard method. J Pharm Res. 2012;5(1):94-9.
29. Nathi R, Rao SS, Sahoo S, Sunkara N, Mohan VR. Stability indicating rp-hplc method development and validation of efavirenz in bulk and pharmaceutical dosage form. Int J Pharm Biol Sci. 2017;7(2):223-9.
30. Kumar G, Sharma S, Shafiq N, Pandhi P, Khuller GK, Malhotra S. Pharmacokinetics and tissue distribution studies of orally administered nanoparticles encapsulated ethionamide used as potential drug delivery system in management of multi-drug resistant tuberculosis. Drug Deliv. 2011;18(1):65-73.
doi: 10.3109/10717544.2010.509367, PMID 20735202.
31. Gautam N, Roy U, Balkundi S, Puligujja P, Guo D, Smith N, *et al.* Preclinical pharmacokinetics and tissue distribution of long-acting nanoformulated antiretroviral therapy. Antimicrob Agents Chemother. 2013;57(7):3110-20. doi: 10.1128/AAC.00267-13, PMID 23612193.
32. Lee SG, Lee J, Kim KM, Lee KI, Bae YS, Lee HJ. Pharmacokinetic study of NADPH oxidase inhibitor Ewha-18278, a pyrazole derivative. Pharmaceutics. 2019;11(9):2-8. doi: 10.3390/pharmaceutics11090482, PMID 31533299.
33. Kushwaha HN, Mohan NK, Sharma AK, Singh SK. Pharmacokinetic study and

bioavailability of a novel synthetic trioxane antimalarial compound 97/63 in rats. Malar Res Treat. 2014;2014:759392. doi: 10.1155/2014/759392, PMID 25302132.

34. Gera S, Talluri S, Rangaraj N, Sampathi S. Formulation and evaluation of naringenin nanosuspensions for bioavailability enhancement. AAPS PharmSciTech. 2017;18(8):3151-62. doi: 10.1208/s12249-017-0790-5, PMID 28534300.
35. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1(2):87-93. doi: 10.4103/0976-500X.72350, PMID 21350616.
36. Kumar P, Lakshmi YS, Kondapi AK. An oral formulation of efavirenz-loaded lactoferrin nanoparticles with improved biodistribution and pharmacokinetic profle. HIV Med. 2017;18(7):452-62. doi: 10.1111/hiv.12475, PMID 28000390.
37. Gaur PK, Mishra S, Bajpai M, Mishra A. Enhanced oral bioavailability of efavirenz by solid lipid nanoparticles: *in vitro* drug release and pharmacokinetics studies. BioMed Res Int. 2014;2014:363404. doi: 10.1155/2014/363404, PMID 24967360.
38. Huang J, Gautam N, Bathena SPR, Roy U, McMillan JE, Gendelman HE, *et al*. UPLC-MS/MS quantifcation of nanoformulated ritonavir, indinavir, atazanavir, and efavirenz in mouse serum and tissues. J Chromatogr B. 2011;879(23):2332-8. doi:10.1016/j.jchromb.2011.06.032.
39. Savic IM, Nikolic VD, Savic IM, Nikolic LB, Stankovic MZ. Development and validation of a new RP-HPLC method for determination of quercetin in green tea. J Anal Chem. 2013;68(10):906-11. doi: 10.1134/S1061934813100080.

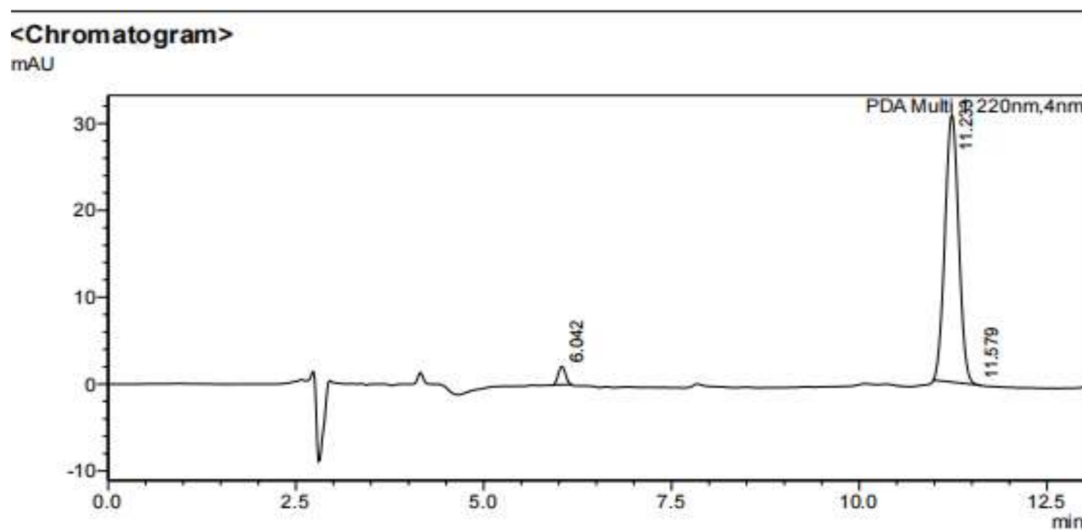


**Figure 1: HPLC chromatogram of Niclosamide**.

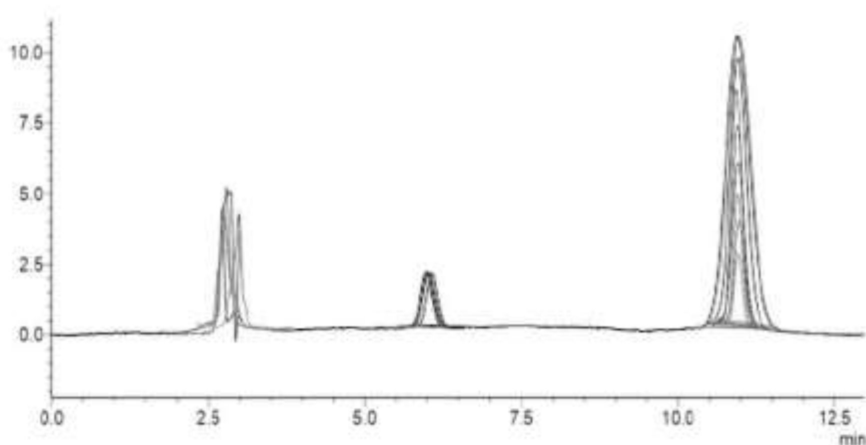


**Figure 2: Overlay chromatogram**

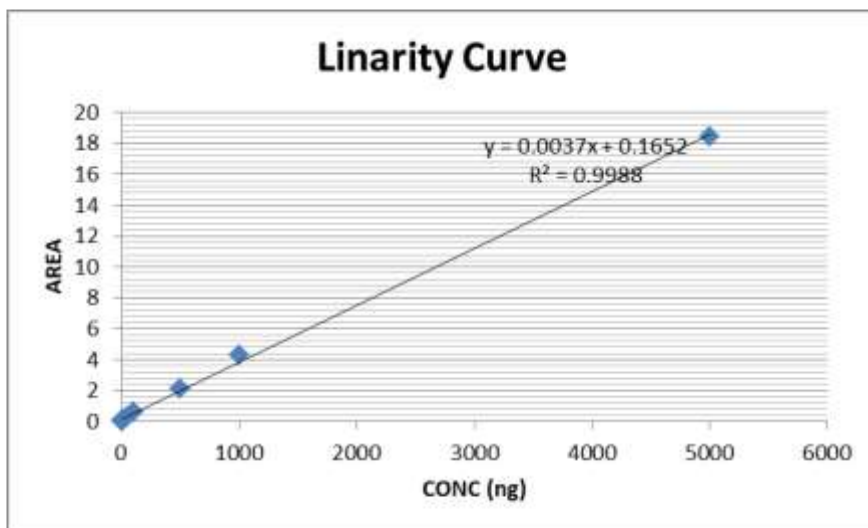


Figure 3: Linearity Curve of NIC

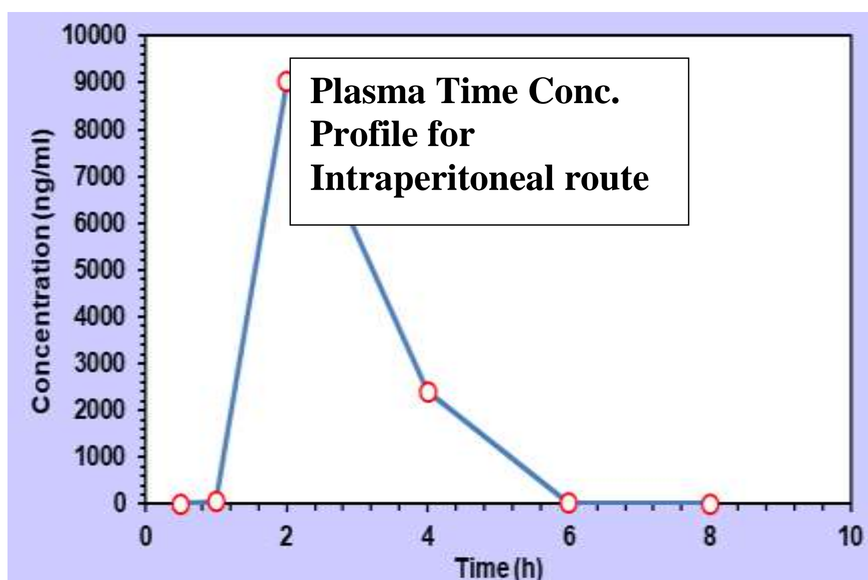


Figure 4: Time vs concentration graph of Intra peritoneal route of administration.

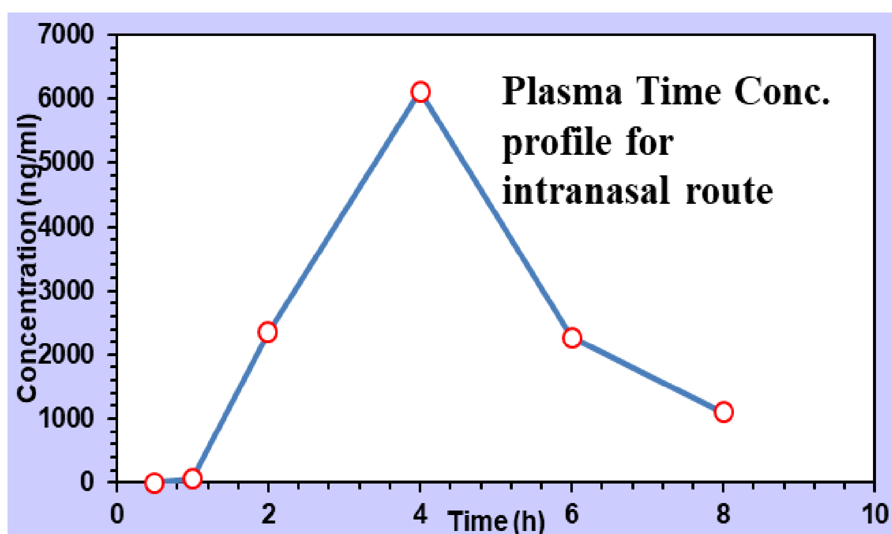


Figure 4: Time vs concentration graph of Nasal route of administration.

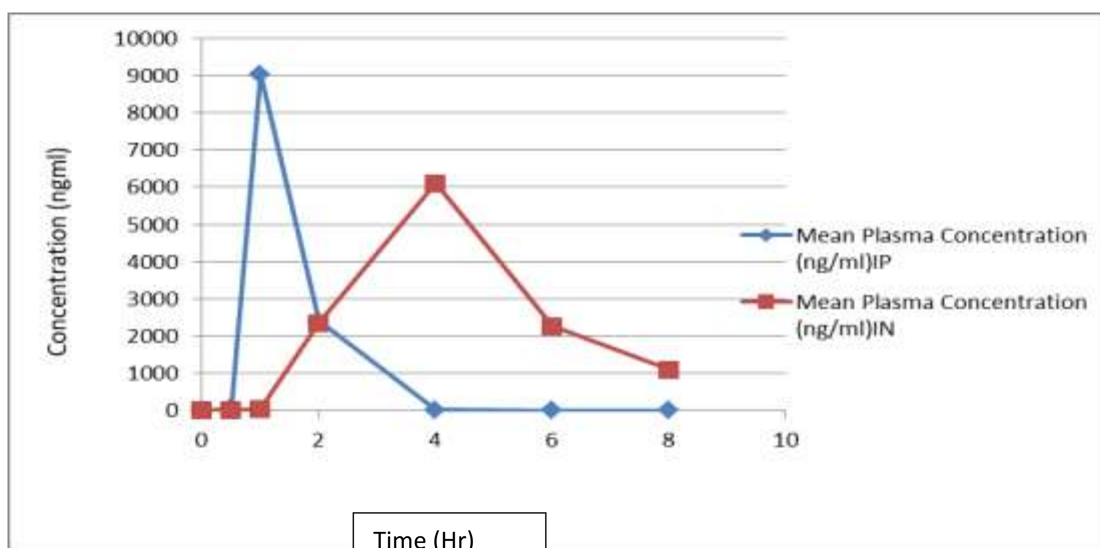


**Figure 5: Graph of Time vs concentration graph of Intranasal and Intraperitoneal route of administration.**

**Table 1: System Suitability Parameters for Resolution of NIC and IS (IBP).**

| Drug | Retention time | Height | Area | HETP | Theoretical plates | Tailing Factor |
|---|---|---|---|---|---|---|
| IBP | 5.995± 0.021 | 2436±27. 48 | 25072± 0.055 | 22.579± 0.031 | 6643± 18.34 | 1.074±0.018 |
| NIC | 11.182± 0.014 | 13175±36 .13 | 168642±0.062 | 11.039± 0.023 | 13588±19.37 | 0.996±0.025 |

**Table 2: Precision and Accuracy parameters for NIC.**

| Sr. No. | Parameters | Values |
|---|---|---|
| 1 | LLOQ | 1.0 ng |
| 2 | LQC | 2.5 ng |
| 3 | MQC | 2500 ng |
| 4 | HQC | 4000 ng |
| 5 | ULOQ | 5000 ng |

**Table 3: Bioanalytical method validation parameters.**

| **Sr. No.** | **Parameters** | **Values** |
|---|---|---|
| 1 | Extraction recovery | 50 ng/ml - 91.12- 93.55 %<br>500 ng/ml - 93.55– 105 %<br>5000 ng/ml - 97.84– 103.56%<br>(Deviation should be less than 15 % ) |
| 2 | Percentage coefficient variation (% RSD) | Interday precision - 1.07 to 6.90 %.<br>Intra-day precision - 1.99 to 6.21 %.<br>(Deviation should be less than 15 % ) |
| 3 | Robustness | Less than 2% relative standard deviation in all parameters (Deviation should be less than 5 % ) |
| 4 | Sample Stability<br>10 days at 4 °C<br>4 freeze– thaw cycles<br>24 h at ambient temp. | The difference between the stability sample (SS) and fresh sample (FS) was found to be <5%.<br>(Deviation should be less than 15 % ) |

**Table 4: Pharmacokinetic data for NIC in plasma after intra peritoneal and nasal route of administration.**

| **Sr. No.** | **Parameters** | **Intra peritoneal route** | **Intranasal route** |
|---|---|---|---|
| 1 | Cmax (ng/ml) | 9031±0.003 | 6109±0.0026 |
| 2 | tmax (h) | 2 | 4 |
| 3 | t1/2 (h) | 0.55845±0.0015 | 1.617186±0.0017 |
| 4 | MRT (h) | 2.5630±0.0013 | 4.94104±0.011 |
| 5 | $AUC_{0-t}$ (ng/ml)h | 18462.75±28.57 | 21405.84±26.175 |
| 6 | AUMC (ng/ml)h 199 | 46845.881±121.03 | 118447.9± 116.26 |

| 7 | Cl (ng/ml)/min | 0.0010827±0.00013 | 0.001043±0.00011 |
|---|---|---|---|

**Table 5: Drug accumulation study in lungs by intranasal administration.**

| Sr. No. | Time (Hr) | Amount of drug (µg) |
|---|---|---|
| 1 | 2 | 6.8 |
| 2 | 4 | 6.2 |
| 3 | 6 | 5.1 |