© IJARW | ISSN (O) - 2582-1008
July 2020 | Vol. 2 Issue. 1
www.ijarw.com

A REVIEW ON NITAZOXANIDE ANALYTICAL METHOD DETERMINATION

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ABSTRACT

Nitazoxanide (NTZ) is an antibiotic that has broad spectrum antiviral and anti-parasitic activity. Its efficacy wasreported against a broad range of parasites, including Entamoeba histolytica, Cryptosporidium parvum, Giardia lamblia, Trichomonas vaginalis, Isospora belli, Ascaris lumbricoides, Taenia saginata, Taenia solium. This article aims to study accurately about various analytical methods used for determination of Nitazoxanide without excipients and in marketed product as well.

The various analytical techniques used are spectrophotometry, electrochemical methods, capillary electrophoresis, high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), gas chromatography

9GC0 and liquid chromatography-mass spectrophotometry (LC-MS).

Keyword: Nitazoxanide, antiviral, antiprotozoal, anthelminthic, Analytical methods

1. INTRODUCTION

Nitazoxanide (NTZ) is an antibiotic that has broad spectrum antiviral and anti-parasitic activity. Initially it was developed as a veterinary anthelminthic with activity against intestinal nematodes, cestodes and trematodes and was approved by the US Food and Drug Administration (FDA) in 2002 for use in human beings. NTZ is found be 99% bound to plasma protein and available as tablet and oral suspension in marketed formulations.

Structure

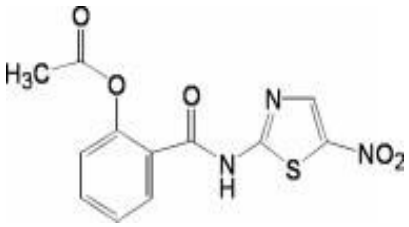

Fig. 1: Structure of Nitazoxanide

NTZ is a nitro-thiazole benzamide compound which is light yellow to pink crystalline powder and is insoluble in water and poorly soluble in ethanol. It's molecular mass of 307.283 g/mole and molecular formula of C12H9N3O5S.Its chemical name as per IUPAC is 2-acetyloxyl-N-(5-nitro-2-thiazolyl) benzamide (Fig. 1).

1.1. Solubility of NTZ:

According to (BCS) Biopharmaceutical Classification System NTZ is placed in Class IV i.e., low solubility and low permeability. It is poorly soluble in ethanol, practically insoluble in water and slightly soluble in acetone as well as chloroform (CHCl3) and is very slightly soluble in methanol. The melting point of NTZ is 202°C.

1.2. Sample Preparation

Sample preparation is an essential and vital used during analytical procedures. It is found that approximately 30% errors were contributed from sample analysis during sample preparation.

The diluents used for the analytical determination of NTZ includes (ACN) Acetonitrile (0.2M), Potassium dihydrogen phosphate (70:30) with an adjustment of pH 3.0 by o-phosphoric acid, HCl 1N, ammonium acetate, CHCl3, and ammonia solution.

The solvents used for NTZ analysis are: 1,4-dioxane, dimethylformamide (DMF), ACN and ethanol.

The sample of NTZ was extracted from various biological membranes like plasma, serum, kidney, urine and brain by the technique of deproteination with ACN, ethanol followed by centrifugation.

1.3. Mechanism of Action

NTZ disrupts energy metabolism in anerobic microbes by inhibition of pyruvate ferredoxin oxidoreductase (PFOR) enzyme cycle. It also induces lesions in cell membrane and depolarizes the mitochondrial membrane. The DNA- derived PFOR protein sequence of Cryptosporidium parvum appears to be similar to that of Giardia lamblia. It also exhibits inhibitory effect on tumor cell progression by altering drug detoxification (glutathione-S-transferase P1), unfolded protein response, anti-cytokines activity and c-Myc inhibition. After administration, it is converted to tizoxanide and tizoxanideglucuronideas active metabolites.

Uses

1. It is most effective for cryptosporidium parvum infection, which causes diarrhea in children and in AIDS patients.
2. It is also indicated in the treatment of giardiasis and in amoebic dysentery as luminal amebicide.

2. ANALYTICAL METHODS

2.1. Spectrophotometry:

In the literature, 9 methods were reported for the estimation of NTZ using spectrophotometry, of which 7 methods are for determining NTZ alone, whereas the remaining are for quantifying NTZ in combination with other drugs substance.

Table 1 shows the summary of the reported spectroscopic methods indicating the basic principle, λ max, solvent, limit of detection (LOD) and limit of quantification (LOQ).

Compounds	**Method**	**λ max (nm)**	**Solvent**	**LOD (µg/ml)**	**LOQ (µg/ml)**
NTZ	Hyposchromic shift-based method	343.5	Methanol:0.1M citric acid(80:20)	0.12	0.39
NTZ in dosage form	Spectrophotometry	238.3	Acetonitrile: water (9:1)		
NTZ	Spectrophotometry	732	1ml ferric chloride (1%) and 2ml MBTH (0.1%)	0.1147	0.3824
NTZ	Simultaneous Equations Method	218.5	Ferric chloride	0.7653	0.8796
NTZ	First derivative spectroscopy	277	Ferric chloride	1.2374	1.1134
NTZ	Second derivative spectroscopy	260,314	Ferric chloride	1.6543	1.2467
NTZ	spectrophotometry	344	ethanol	0.907	0.299
NTZ, Ofloxacin	Q-analysis method	346.36	1NHCl in methanol		
NTZ, Ofloxacin	Vierodts method	346.3, 296.49	1N HCl in methanol		

2.2. Electrochemical methods:

The determination of electrochemical behavior of NTZ was studied using voltametry. The authors used to hang mercury electrode as sensor for the NTZ in Britton-Robinson universal buffer of pH values 2 to 11.

Methods used for voltammetric determination are cyclic and square-wave voltammetry, Cyclic voltammetric Square wavecathodic adsorptive stripping voltammetry (SW-CAdSV) and Differential pulse cathodic adsorptive stripping voltammetry (DP-CAdSV), linear sweep cathodic adsorptive stripping voltammetry (LS-CAdSV), differential pulse polarography (DPP).

Compounds	Method	Linear response	Correlation coefficient	LOD (µg/ml)	LOQ (µg/ml)
NTZ in human serum	LS-CAdSV DP-CAdSV SW-CAdSV	3×10-9 to 2×10-7 mol L-1, 5×10-9 to 1×10-7mol L-1, 1×10-9 to 1×10-7mol L-1	0.985, 0.990, 0.999	9x10-10, 1.5×10-9, 3×10-10mol L-1	3×10-9, 5×10-9, 1×10-9mol L-1
NTZ in human urine	DP-CAdSV, SW-CAdSV	1×10-9 to 1×10-8mol L-1	0.9965, 0.9985	2.078×10-10, 1.365×10-10 molL-1	4.551×10-10, 6.926×10-10 mol L-1
NTZ in human breast milk	DP-CAdSV, SW-CAdSV	1×10-8 to1×10-9 mol L-1	0.9999, 0.9993	0.601×10-10 mol L-1, 0.718×10-10 mol L-1	2.00×10-10 mol L-1, 2.393×10-10 molL-1
NTZ in bulk form	DP-CAdSV, SW-CAdSV	1x10-9 to 1x10-8 mol L- 1	0.9961, 0.999	1.878x10-10 mol L-1, 1.078x10-10 mol L-1	6.262 x10-10 mol L-1, 3.595x10-10mol L-1

Shital Gandhi et al (19) developed a simple, sensitive and highly selective electrochemical method to determine NTZ and ofloxacin on aqueous media likely BRITTON- ROBINSON buffer having ph8 on HMDE with the help of DPP. With the help of DPP used for separation of 936mV between peak oxidation potentials present in binary mixtures for nitazoxanide and ofloxacin were obtained. For the determination of NTZ and ofloxacin quantification limits were 0.083µg/ml and 0.208µg/ml.

2.3. Chromatographic techniques

HPLC:

- Biological samples:

A high-performance liquid chromatographic method was optimized and validated for the determination of desacetyl NTZ (tizoxanide), human plasma, breast milk and urine are the main active metabolites of NTZ.

CN column with acetonitrile consisting of mobile phase has proposed. Acetonitrile

Composed 12mm ammonium acetate- diethyl amine of ratio 30:70:1 (v/v/v) and buffered pH 4.0 along with acetic acid, having flow rate of 1.5/min.

Finally with the help of uv detection, quantitation was achived at 260nm by using nifuroxazide which is considered as internal standard.

The preparation of human urine sample was done by SONICATION of 0.1nm sodium hydroxide for 15min and further neutralized with 0.1M hcl.

The results of LOD and LOQ were 3.56_1022 and 11.87_1022 respectively.

Further preparation of human breast milk was completed by mixing and homogenizing NIFUROXAZIDE with orthophosphoric acid, and their LOD and LOQ found 4.47 and 14.9 respectively.

Preparation of human plasma by mixing acetonitrilw, NF 0.1M sodium hydroxide and further sonicated for 15min, then neutralized by the help of 0.1M HCL. LOD and LOQ was found 4.8 and 16.254 respectively.

2.4. Pharmaceutical samples:

Analytical methods for the determination of isoniazid in pharmaceutical dosage forms using HPLC

Mobile phase	Column	Detection	λ max (nm)	Flow rate	LOD	LOQ
Study aim: Quantification of NTZ in presence of its alkaline degradation product						
acetonitrile: 50 mM ammonium acetate buffer (50:50, v/v,pH 5.0 adjusted with acetic acid)	Inertsil C8-3 column (150 × 4.6 mmi.d.)	UV	298 nm	1 mL/min.	0.0410	0.1242 µg/mL
Study aim: HPLC method of isocrtic reverse phase for the simultaneous determination of NTZ and OFLOXACIN obtained from combined dosage from						
2.0gm sodium dihydrogen phosphate and 5M of triethylamine are mixed into 500mL Milli Q water and pH was adjusted to 4.5 by orthophosphoric acid	Phenomenex Luna C18 reversed-phase column	UV	305 nm	1.5 mL/min	0.174µg/ml and 0.21µg/ml	0.034µg/ml and 0.08µg/ml
Study aim: Determination of NTZ in oral suspension dosage form						
Mixture of acetonitrile: ammonium dihydrogen phosphate buffer (0.075 M) inthe ratio 45:55 (% v/v) adjusted to pH 3.0 withorthophosphoric acid	Qualisil BDS C18 (4.6× 250mm, 5µ)	UV	240 nm	1.5 mL/min	0.25	0.77
Study aim: Simultaneous Estimation of NTZ and Ofloxacin from Tablet Dosage						
acetonitrile: potassium dihydrogen ortho phosphate (pH 4.5, 10mM) (60:40 v/v)	Princeton SPHER C18 column (250mm×/4.6 mm i.d.)	UV	265nm	1.0ml/min		

T. Sakamoto et al (27) developed a simple and rapid determination method for NTZ using reverse-phase HPLC and Ultra Performance Liquid Chromatography(UPLC).

Mobile phase consisted of a mixture of phosphate buffer (pH 6.0) and acetonitrile HPLC System-HPLC Shimadzu Class-VP HPLC system. A Waters symmetry C18 Column (150 mm_4.6 mm I.D., 5 mm particle size, Waters Co., Milford, MA, USA),

The UPLC SYSTEM- A waters ACQUITY UPLC system. C18 (50 mm_2.1 mm I.D., 1.7 mm particle size, Waters Co., MA, USA)

The tailing factor of the NTZ peak was 2.0 for HPLC and 1.2 for UPLC, respectively. The retention times of IS (nifuroxazide) and NTZ were 22.1 and 24.8 min for HPLC, and 3.2 and 3.5 min for UPLC, respectively. The correlation coefficients were 0.9988 (HPLC) and 0.9963 (UPLC). The RSDs of quantitative values of sample solution were calculated to be 4.06% to 4.64% for HPLC and 0.15% to 0.36% for UPLC.

GC:

The residual solvents in NTZ was developed by Jiang Shan et al (28) were separated by a DM-WAX column (30 m×0.25 mm,0.5μm) with an FID detector. The injector temperature and the detector temperature were set at 200℃and 250℃, respectively. The containers of head-space injector were in equilibrium at 80℃for 30 min. N, N- Dimethylformamide was used as the solvent. The detected solvents were separated completely. A good linearity of the two solvents was obtained within the range of 250-750μg/ml(r=0.9991) and 30-90μg/ml (r=0.9991), respectively. The average recovery of acetone and dichloromethane was 99.15%and 99.18%with RSD of 2.17%and 2.97%(n=9), respectively.

LC-MS:

LC-MS method was developed by Zhanzhong Zhao et.al developed a sensitive and specific method for the identification of NTZ metabolites in goat feces by liquid chromatography–electrospray ionization tandem mass spectrometry with negative ion mode was developed. After extraction procedure the pretreated samples were injected on an XTerra MS C8 column with mobile phase (0.2 mL min−1) of acetonitrile and 10 mM ammonium acetate (adjusted to pH 2.5 with formic acid) followed by a linear gradient elution, and detected by MS–MS. Identification and structural elucidation of the metabolites were performed by comparing their retention times (R t), full scan, product ion scan, precursor ion scan and neutral loss scan MS–MS spectra to those of the parent drug or other available standard. The parent drug (NTZ) and its deacetyl metabolite (tizoxanide) were found in goat feces after the administration of a single oral dose of 200 mg kg−1 of NTZ. Tizoxanide was detected in goat feces for up to 96 h after ingestion of NTZ.

Huang X et al(30) utilized a hybrid linear ion trap/Orbitrap mass spectrometer providing a high mass resolution and accuracy was used to investigate the metabolism of NTZ in rats, pigs, and chickens. The results revealed that acetylation and glucuronidation were the main metabolic pathways in rats and pigs, whereas acetylation and sulfation were the major metabolic pathways in chickens, which indicated interspecies variations in drug metabolism and elimination. With the accurate mass data and the characteristic MS(n) product ions, we identified six metabolites in which tizoxanide and hydroxylated tizoxanide were phase I metabolites and

tizoxanide glucuronide, tizoxanide glucose, tizoxanide sulfate and hydroxyl tizoxanide sulfate were phase II metabolites. Hydroxylated tizoxanide and tizoxanide glucose were identified for the first time. All the comprehensive data were provided to make out the metabolism of NTZ in rats, pigs and chickens more clearly. The photodegradation of NTZ was studied by M.D. Malesuiketal in order to investigate the degradation kinetics of this drug. The analyses of the degraded samples were performed by a stability-indicating liquid chromatographic method. The column utilized was a Phenomenex (Torrance, CA) Synergi Fusion C18 column (250mm, 4.6 mm, i.d., 4μm particle size) coupled to a C18guard column(4.0mm3.0mm, i.d., 4 μm). a mobile phase of ophosphoric acid 0.1% (v/v) (pH 6.0 adjusted by addition of triethylamine)–acetonitrile (45:55, v/v) run at a flow rate of 1.0mL/min and using PDA detection at 240 nm. The light source was an UVC – 254 nm30Wlamp (Philips,Amsterdam, Holland) fixed to a chamber in a horizontalposition.

Degradation Rate Constant (k), Half-life (t1/2), and t90 for NTZ in Pharmaceutical Formulations Solutions Submitted to Photodegradation and Determined by LC Method.

Dosage forms	k/min	$t_{1/2}$ (min)	t_{90} (min)
Tablets	4.81,10–2	201. 56	40.30
Powder for oral suspension	5.38, 10–2	183.83	36.75

HPTLC:

A new simple, rapid, and selective high-performance thin-layer chromatographic (HPTLC) method with metronidazole as the internal standard has been developed by Salvador Namuret alfor analysis of tizoxanide (a metabolite of nitazoxanide) in human plasma. The analyte was extracted from human plasma by cation-exchange solid-phase extraction (SPE). In HPTLC the stationary phase was silica gel 60F254 and the mobile phase was toluene-ethyl acetate-acetic acid 6.2:13.4:0.4 (v/v). UV detection and quantification were performed at 313 nm for the internal standard and 410 nm for tizoxanide. Data were fitted to a quadratic mathematical function by polynomial regression. The working range was 400–16000 ng mL −1. The method was validated for accuracy and precision. The average recovery was85.5%.

A validated stability indicating HPTLC was developed by CL Gopuetal for determination of nitazoxanide in bulk and in formulation. They carried out separation in TLC alumina plates precoated with silica gel 60F254using mixture of ethyl acetate-toluene-methanol (3.9:6.1:1 and 4.1:5.9:1 v/v/v) as mobile phase. The detection of spot was carried out by using UV detector at 350 nm. The linearity of calibration curve was found to be between 400- 1600 ng per spot.

3. CONCLUSION

A large number of techniques are available for the estimation of nitazoxanide in pharmaceutical formulations and biological samples. The survey of analytical data revealed that HPLC methods are predominant for the estimation of drug alone or in combination with other drugs in various formulation types. So, for the precise and accurate separation of nitazoxanide in various formulations recommended method of analysis includes HPLC with UV detector as it provides faster analysis time and has more separation selectivity than most other availabletechniques.

This review carried out an overview of the state-of-art analytical methods for the determination of Nitazoxanide in different formulations using various analytical techniques.

ACKNOWLEDGEMENT: XYZ

CONFLICT OF INTEREST: - NONE

4. DECLERATION

I hereby declare that the work incorporated in the present project report entitled

"NITAZOXANIDE: A REVIEW ON ITS ANALYTICAL METHOD DETERMINATION" is my own work and is original. This work (in part or full) has not been previously submitted to any University for the award of Degree

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