Reported Analytical Methods for Nitazoxanide: A Review

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ABSTRACT:
Antibiotic nitazoxanide (NTZ) has significant antiviral and antiparasitic action. Various parasites, including Entamoeba histolytica, Cryptosporidium parvum, Giardia lamblia, Trichomonas vaginalis, Isospora belli, Ascaris lumbricoides, Taenia saginata, and Taenia solium, have been documented to be adaptable to it. In this paper, multiple methods of analysis for determining nitazoxanide without excipients and in marketed products are carefully studied. Spectrophotometry, electrochemical methods, capillary electrophoresis, high performance thin layer chromatography (HPTLC), gas chromatography (GC), and liquid chromatography-mass spectrophotometry (LC-MS) are among the different analytical techniques employed.

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

INTRODUCTION:
Having a wide range of antiparasitic and antiprotozoal activities is the novel compound nitazoxanide (NTZ). Its chemical name is 2-acetyloxyl-N-(5-nitro-2-thiazolyl) benzamide, and it is a nitrothiazole derivative.⁴ Intestinal nematodes, cestodes, and trematodes are all targets of its first veterinary anthelminthic development. The US Food and Drug Administration (FDA) authorised NTZ for use in humans in 2002.²
It is utilised to treat helminthiasis as well as intestinal protozoal infections. In patients with impaired immune systems, such as those who have AIDS or HIV infection, it is also used to treat cryptosporidiosis and diarrhoea brought on by Giardia lamblia (4, 5, 6, 7, 8). NTZ is thought to have antipROTOzoal properties through interfering with an enzyme-dependent electron transfer pathway known as pyruvate ferredoxin oxidoreductase (PFOR), which is crucial for anaerobic energy metabolism. Studies have demonstrated that, in the absence of ferredoxin, the PFOR enzyme from Giardia lamblia directly lowers nitazoxanide through the transfer of electrons. The protein sequence of PFOR derived from DNA in Giardia lamblia and Cryptosporidium parvum seems to be identical.4

Nitazoxanide is a crystalline powder which is light yellow or pink in appearance. It is difficult to dissolve in ethanol and does not dissolve in water. Its chemical formula is C12H9N3O5S (9), and its molecular weight is 307.283 g/mole. It transforms into the active metabolites tizoxanide and tizoxanide glucuronide after consumption. More than 99% of NTZ in plasma is protein-bound. It is available in the market as tablets and oral suspension.6

SOLUBILITY PREPARATION:

SOLUBILITY:

According to Biopharmaceutical Classification System (BCS), nitazoxanide is a class IV drug (low solubility and low permeability). It is slightly soluble in acetone, chloroform and very slightly soluble in methanol and practically insoluble in water. The melting point of nitazoxanide is 202°C.5

SAMPLE PREPARATION STRATEGIES:

Sample preparation is the integrated part of analytical methodology, and it was reported that approximately about 30% errors contributed from sample analysis was due to sample preparation.6 Among the various diluents used for the analysis of nitazoxanide are acetonitrile, 0.3M potassium dihydrogen phosphate: methanol 70:10:20 (pH 3.5 adjusted with o-phosphoric acid), methanol, ethanol, sodium hydroxide, ammonia acetate, glacial acetic acid, chloroform, and ammonia solution. Dimethyl formamide (DMF), 1,4-dioxane, acetonitrile, and ethanol are the solvents employed. By deproteinizing the biological matrices such as plasma, serum, urine, liver, kidney, and brain with acetonitrile and ethanol, followed by centrifugation, nitazoxanide was extracted from them.7

Various techniques have been documented in the literature for assessing the concentration of NTZ (nitazoxanide) using spectrophotometry. These methods can be categorized into two groups: some are specifically designed for the sole determination of NTZ, while others are meant for quantifying NTZ in combination with other drug substances.7

MECHANISM OF ACTION

By inhibiting the pyruvate ferredoxin oxidoreductase (PFOR) enzyme cycle, NTZ interferes with anaerobic bacteria’ ability to metabolise energy. It also induces lesions in cell membrane and depolarizes the mitochondrial membrane.8 The DNA-obtain PFOR protein sequence of Giardia lamblia and Cryptosporidium parvum appear to be identical to one another. It also exhibits inhibitory effect on cancer cell progression by altering medicine detoxification (glutathione- S- transferase P1), unfolded protein response, anti-cytokines activity and c-Myc inhibition. After administration, it's converted to tizoxanide and tizoxanideglucur on ideas active metabolites.9

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.
SPECTROPHOTOMETRY:

There are nine methodologies for estimating NTZ using spectrophotometry that have been described in the literature; seven of these methods are for quantifying NTZ alone, and the other three are for estimating NTZ along with other medicines.  

Table 1 shows the overview of the reported spectroscopic techniques that includes the fundamental principle, λ max, solvent, limit of detection (LOD), and limit of quantification (LOQ).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Methods</th>
<th>λ max (nm)</th>
<th>Solvent</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTZ</td>
<td>Hyposchromic shift based method</td>
<td>343.5</td>
<td>Methanol :0.1M citric acid (80:20)</td>
<td>0.12</td>
<td>0.39</td>
<td>10</td>
</tr>
<tr>
<td>NTZ in dosage form</td>
<td>spectrophotometry</td>
<td>238.3</td>
<td>Acetonitrile: water (9:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTZ</td>
<td>spectrophotometry</td>
<td>732</td>
<td>1ml ferric chloride (1%) and 2ml MBTH (0.1%)</td>
<td>0.1147</td>
<td>0.3824</td>
<td>11</td>
</tr>
<tr>
<td>NTZ</td>
<td>The simultaneous equation method</td>
<td>218.5</td>
<td>Ferric chloride</td>
<td>0.7653</td>
<td>0.8796</td>
<td></td>
</tr>
<tr>
<td>NTZ</td>
<td>First derivative spectroscopy</td>
<td>277</td>
<td>Ferric chloride</td>
<td>1.2374</td>
<td>1.1134</td>
<td>12</td>
</tr>
<tr>
<td>NTZ</td>
<td>Second derivative spectroscopy</td>
<td>260,314</td>
<td>Ferric chloride</td>
<td>1.6543</td>
<td>1.2467</td>
<td></td>
</tr>
<tr>
<td>NTZ</td>
<td>spectrophotometry</td>
<td>344</td>
<td>ethanol</td>
<td>0.907</td>
<td>0.299</td>
<td></td>
</tr>
<tr>
<td>NTZ, Ofloxacin</td>
<td>Q-analysis method</td>
<td>346.36</td>
<td>1N HCL in methanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTZ, Ofloxacin</td>
<td>Vierodts method</td>
<td>346.3,296.49</td>
<td>1N HCL in methanol</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>NTZ, Ofloxacin</td>
<td>Dual wavelength method</td>
<td>333.6, 359.2, 302.4, 289.2</td>
<td>1N HCL in methanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTZ, Ofloxacin in tablet dosage form</td>
<td>Simultaneous estimation using RP-HPLC</td>
<td>300</td>
<td>Acetonitrile, methanol, 0.4M CITRIC ACID</td>
<td>0.3788 and 0.0929 g/ml</td>
<td>1.1479 and 0.2816 g/ml</td>
<td>15</td>
</tr>
</tbody>
</table>

Electrochemical techniques:

The determination of electrochemical actions of NTZ was studied using voltametric. The litterateurs used suspending mercury electrode as detector for the NTZ in Britton- Robinson universal buffer of pH values 2 to 11. techniques used for voltametric determination are cyclic and square- wave voltammetry, Cyclic voltammetric square wave cathodic adsorptive stripping voltammetry (SW- CaDSV) and Differential beat cathodic adsorptive stripping voltammetry (DP- CaDSV), direct reach cathodic adsorptive stripping voltammetry (LS- CaDSV), differential pulse polarography (DPP).

Shital Gandhi and colleagues (Gandhi et al., 16) have devised an uncomplicated, highly sensitive, and selective electrochemical method to simultaneously detect NTZ (nitazoxanide) and ofloxacin in a non-aqueous environment (Britton-Robinson buffer, pH 8.36).16 They employed a hanging mercury drop electrode (HMDE) in conjunction with differential pulse polargraphy (DPP) to achieve this simultaneous determination of the two substances. Using DPP a separation of about 936 mV between the peak oxidation capabilities of nitazoxanide and ofloxacin present in double combinations was obtained. The quantification limits for the contemporaneous determination of NTZ and ofloxacin were0.083 µg/ ml and0.208 µg/ ml.7
<table>
<thead>
<tr>
<th>Compounds in</th>
<th>Method</th>
<th>Linear response</th>
<th>Correlation coefficient</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>pharmaceutical formulation</td>
<td>Cyclic and square-wave voltammetry</td>
<td>20-140 mg/ml</td>
<td>0.9938</td>
<td>5.23 µg/ml</td>
<td>17.45 µg/ml</td>
<td>17</td>
</tr>
<tr>
<td>NTZ in human urine</td>
<td>DP-CA, SW-CA</td>
<td>1×10-9 to 1×10-8 mol L-1</td>
<td>0.9965, 0.9985</td>
<td>2.078×10-10, 1.365×10-10</td>
<td>4.551×10-10, 6.926×10-10</td>
<td>19</td>
</tr>
<tr>
<td>NTZ in human serum</td>
<td>LS-CA, DP-CA, SW-CA</td>
<td>3×10-9 to 2×10-7 mol L-1, 5×10-9 to 1×10-7 mol L-1, 1×10-9 to 1×10-7 mol L-1</td>
<td>0.985, 0.990, 0.999</td>
<td>9×10-10, 1.5×10-9, 3×10-10 mol L-1</td>
<td>3×10-9, 5×10-9, 1×10-9 mol L-1</td>
<td>18</td>
</tr>
<tr>
<td>NTZ in human breast milk</td>
<td>DP-CA, SW-CA</td>
<td>1×10-8 to 1×10-9 mol L-1</td>
<td>0.9999, 0.9993</td>
<td>0.601×1010 mol L-1, 0.718×1010 mol L-1</td>
<td>2.00×10-10 mol L-1, 2.393×10-10 mol L-1</td>
<td>19</td>
</tr>
<tr>
<td>NTZ in bulk form</td>
<td>DP-CA, SW-CA</td>
<td>1×10-9 to 1×10-8 mol L-1</td>
<td>0.9961, 0.999</td>
<td>1.878×1010 mol L-1, 1.078×1010 mol L-1</td>
<td>of 6.262×10-10 mol L-1, 3.595×1010 mol L-1</td>
<td></td>
</tr>
<tr>
<td>NTZ in spiked plasma</td>
<td>Turn-on-type fluorescence resonance energy transfer ecofriendly</td>
<td>0.1 to 0.6 µg/ml</td>
<td>0.9996</td>
<td>0.013 µg/ml</td>
<td>0.038 µg/ml</td>
<td>20</td>
</tr>
<tr>
<td>NTZ in biological matrices</td>
<td>LC-MS/MS</td>
<td>0.53-21.2 ng/ml</td>
<td>0.999</td>
<td>-</td>
<td>0.53 ng/ml</td>
<td>21</td>
</tr>
</tbody>
</table>

CHROMATOGRAPHY:

HPLC:

BIOLOGICAL SAMPLES

Ghada M. and colleagues optimized and validated a high-performance liquid chromatographic (HPLC) system for the precise quantification of desacetyl nitazoxanide (tizoxanide), which is the primary active metabolite of NTZ, in human serum, urine, and breast milk. 22

The proposed system used a CN column with mobile phase conforming of acetonitrile – 12mM ammonium acetate – diethylamine in the proportion of 30700.1 (v/ v/v) and softened at pH4.0 with acetic acid, with a stream rate of 1.5 mL/ min.

Quantitation was achieved with UV observation at 260 nm using nifuroxazide as internal standard.

Human urine sample was prepared by alkalizing with 0.1 M sodium hydroxide sonicated for 15 min and neutralized with 0.1 M hydrochloric acid. The LOD was initiate to 3.56, 1022 be and LOQ was initiate to be11.87, 1022. mortal breast milk sample was prepared by homogenizing and mixing with nifuroxazide and orthophosphoric acid. The LOD was initiate to be4.47 and LOQ was initiate to be14.9. Human plasma was
prepared by mixing with acetonitrile, NF0.1 M sodium hydroxide. It's sonicated for 15min., annulled by0.1 M hydrochloric acid. The LOD4.8 was set up to be and LOQ was set up to be16.24 (23) T. Sakamoto et al developed a simple and rapid determination method for NTZ using reverse-phase HPLC and Ultra Performance Liquid Chromatography (UPLC).24

GC:

The residual solvents in nitazoxanide 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 was adjusted to 200, while the detector temperature was set to 250. 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 ol 250-750μg/ml(r=0.9991) and 30-90μg/ml (r=0.9991), respectively. Acetone and dichloromethane exhibited an average recovery of 99.15% and 99.18%, respectively, with relative standard deviations (RSD) of 2.17% and 2.97% based on nine replicates.

LC-MS:

LC- MS system is a sensitive and specific system for the identification of NTZ metabolites in scapegoat feces by liquid chromatography – electrospray ionization tandem mass spectrometry with negative ion mode was developed. After birth procedure the pretreated samples were fitted on an XTerra MS C8 column with mobile phase(0.2 mL min ̈1) of acetonitrile and 10 mM ammonium acetate( acclimated to pH2.5 with formic acid) followed by a direct grade elution, and detected by MS – MS. Identification and structural explication 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 medicine or other available standard. Following the administration of a single oral dose of 200 mg kg1 of NTZ, the parent medication (NTZ) and its deacetyl metabolite (tizoxanide) were discovered in goat feces. Tizoxanide was detected in goat feces for over to 96 h after ingestion of NTZ.(25)

Huang X and colleagues employed a hybrid linear ion trap/Orbitrap mass spectrometer with high mass resolution and accuracy to study the metabolism of NTZ (nitazoxanide) in rats, pigs, and chickens. Their findings revealed that rats and pigs predominantly underwent acetylation and glucuronidation as the primary metabolic pathways, while chickens exhibited acetylation and sulfation as the major pathways, indicating inter-species variations in drug metabolism and elimination. By utilizing accurate mass data and characteristic MS(n) product ions, the researchers identified six metabolites, including tizoxanide and hydroxylated tizoxanide as phase I metabolites, and tizoxanide glucuronide, tizoxanide glucose, tizoxanide sulfate, and hydroxyl tizoxanide sulfate as phase II metabolites. Notably, hydroxylated tizoxanide and tizoxanide glucose were identified for the first time. These comprehensive findings contribute to a clearer understanding of the metabolism of NTZ in rats, pigs, and chickens. (26)

M.D. Malesuike and colleagues conducted a study to investigate the photodegradation kinetics of NTZ (nitazoxanide). For the analysis of degraded samples, they utilized a stability-indicating liquid chromatographic method. The chromatographic system consisted of a phenomenex (Torrance, CA) Synergi Fusion C18 column (250mm, 4.6 mm i.d., 4μm particle size) coupled to a C18 guard column (4.0mm 3.0mm i.d., 4 μm). The mobile phase used was a combination of 0.1% (v/v) o-phosphoric acid (pH 6.0 adjusted with triethylamine) and acetonitrile in a ratio of 45:55 (v/v). The analysis was performed at a flow rate of 1.0 mL/min, and PDA detection was carried out at 240 nm.

To induce photodegradation, an UVC – 254 nm 30W lamp (Philips, Amsterdam, Holland) was employed and positioned horizontally within a chamber. (27)

The degradation rate constant (k), half-life (t1/2), and t90 values of NTZ in pharmaceutical formulation solutions, subjected to photodegradation, were determined using a liquid chromatography (LC) method. (7)
HPTLC:

Salvador Namur and colleagues developed a novel, efficient, and specific high-performance thin-layer chromatographic (HPTLC) method, employing metronidazole as the internal standard, for the analysis of tizoxanide (a metabolite of nitazoxanide) in human plasma. The analyte was extracted from human plasma using cation-exchange solid-phase extraction (SPE). Silica gel 60F254 served as the stationary phase in HPTLC, while the mobile phase comprised toluene-ethyl acetate-acetic acid in a ratio of 6.2:13.4:0.4 (v/v/v). UV detection and quantification were carried out at 313 nm for the internal standard and 410 nm for tizoxanide. The obtained data were fitted to a quadratic mathematical function using polynomial regression. The method demonstrated a wide working range from 400 to 16,000 ng mL−1, and accuracy and precision were validated, yielding an average recovery of 85.5%. (28)

CL Gopu and colleagues (reference 33) developed a validated stability indicating high-performance thin-layer chromatography (HPTLC) method for the determination of nitazoxanide in both bulk form and formulations. The separation was conducted on TLC alumina plates coated with silica gel 60F254, using a mobile phase consisting of a mixture of ethyl acetate, toluene, and methanol in ratios of 3.9:6.1:1 and 4.1:5.9:1 (v/v/v). Spot detection was performed using a UV detector at 350 nm. The calibration curve demonstrated good linearity within the range of 400 to 1600 ng per spot. (29)

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28. Salvador Namur, Lizbeth Carino, Mario Gonzalez-de la Parra, Development and validation of a high performance thin-layer chromatographic method, with densitometry, for quantitative analysis of tizoxanide (a Metabolite of Nitazoxanide) in human plasma, November 08, 2007.
