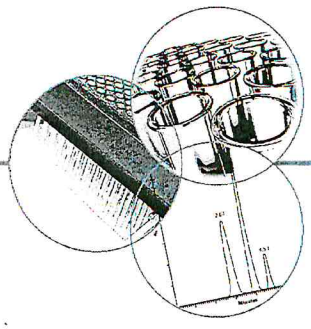
RESEARCH ARTICLE



Development of a method by UPLC–MS/MS for the quantification of tizoxanide in human plasma and its pharmacokinetic application

Background: Nitazoxanide (NTZ) is used for the treatment of gastrointestinal tract colonization by anaerobic bacteria, viruses and other pathogens that represent a major cause of morbidity in Latin America. The aim of the present work was to develop and validate a UPLC–MS/MS method for the selective quantification of tizoxanide (TZN, the major metabolite of NTZ) in human plasma using niclosamide as internal standard; and examine its pharmacokinetic application in healthy volunteers. Nine male subjects received a single oral dose of a NTZ 500-mg tablet under fasting conditions. **Results:** The method was linear between 0.1 and 10 µg/ml and capable of separating signals from free-TZN and those delivered by in-source collision-induced dissociation of TZN-glucuronide, quantifying it with accuracy and precision. Mean maximum plasma concentration was 6.79 µg/ml and was reached at 2.4 h post-dose. **Conclusion:** The method was validated, fulfilling regulatory guidelines. Results suggest low pharmacokinetic variability in the assayed population.

Nitazoxanide (NTZ; 2-(acetyloxy)-*N*-(5-nitro-2-thiazolyl)benzamide; CAS 55981–09–4) is a slightly lipophilic weak base (logP = 1.79; pKa = 6.18) [1]. It is a light yellow crystalline powder that is practically insoluble in water and belongs to the class II biopharmaceutical group (low solubility, high permeability). It was registered in Mexico in 1996 first as an anthelmintic and antiprotozoal agent [2]. The molecular mechanism of NTZ appears to be noncompetitive inhibition of pyruvate-ferrodoxin oxidoreductases, enzymes that participate during energy generation through oxidative decarboxylation of pyruvate to acetyl coenzyme A in parasites [3].

New clinical interest has arisen in the use of NTZ for the treatment of gastrointestinal tract colonization by anaerobic bacteria, viruses and other pathogens that represent a major cause of morbidity in Latin America, Africa and Southeast Asia [4]. Recent insights on NTZ employed in the management of hepatic amebiasis [5], ascariasis [6], giardiasis [7] and echinococcosis [8] are being generated. Special attention is focused on its use as adjuvant with pegIFN in the treatment of hepatitis C [9] and norovirus gastroenteritis in immunosuppressed populations [10], suggesting different NTZ pharmacodynamics on intra- versus extra-cellular pathogens.

NTZ is administered orally and is partially absorbed from the gastrointestinal tract. The nonabsorbed portion acts in the luminal space on cavity parasites, while NTZ in systemic circulation is rapidly and completely hydrolyzed by stearases to **deacetyl-nitazoxanide** or **tizoxanide** (TZN; CAS 173903-47-4) (FIGURE 1), the main metabolite in humans. This is a more lipophilic molecule (logP = 2.91; calculated by using ChemSketch version 12 software; Advanced Chemistry Developments, Inc., Toronto, Canada) that preserves the activity of the parent drug and is well distributed in other tissues. TZN is then widely glucuronized in the liver (increasing its hydrophilicity, logP = -0.6), and excreted by bile (66%) and urine (31.5%). When TZN-glucuronide is secreted again into the intestine, it is entirely hydrolyzed and only free-TZN is detected in feces. After a single oral dose of NTZ, peak plasma concentration (C_{max}) of TZN is reached at approximately 2 h and is removed from the blood with an elimination half-life of approximately 1.75 h [11].

Special care must be taken during TZN measurement due to the possibility of glucuronide hydrolysis during extraction and/or **in-source collision-induced dissociation** (CID) during MS detection [12]. To our knowledge, few analytical methods have been reported for selective TZN quantification. One of the first previously published techniques extracts TZN from human plasma by direct precipitation and analysis through LC with ultraviolet detection

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