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White Paper

Theme: Best Practices for Bioanalytical Methods: Recommendations from the Global Bioanalysis Consortium
Guest Editors: Binodh DeSilva and Philip Timmerman

Small Molecule Specific Run Acceptance, Specific Assay Operation, and Chromatographic Run Quality Assessment: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Teams

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Abstract. Consensus practices and regulatory guidance for liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) assays of small molecules are more aligned globally than for any of the other bioanalytical techniques addressed by the Global Bioanalysis Consortium. The three Global Bioanalysis Consortium Harmonization Teams provide recommendations and best practices for areas not yet addressed fully by guidances and consensus for small molecule bioanalysis. Recommendations from all three teams are combined in this report for chromatographic run quality, validation, and sample analysis run acceptance.

KEY WORDS: bioanalytical assay validation; LC-MS/MS; sample analysis; small molecule.

INTRODUCTION

This summary is based on the discussion among scientific representatives from Mexico, Brazil, Argentina, Japan, India, the UK, Germany, Switzerland, and the USA serving on the Global Bioanalytical Consortium S1, S2, and S3 harmonization teams (HT). These are recommended best practices for specific laboratory operations associated with run acceptance, assay operation, and chromatographic run quality assessment not addressed clearly or not addressed at all in current regulatory guidances or guidelines. In some cases, the team assessing an activity intentionally chose to recommend practices based on good scientific principles that are different from what current regulatory guidances may

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Bioequivalence Evaluation of Two Brands of Meloxicam Tablets (Promotion® and Mobicox®): Pharmacokinetics in a Healthy Female Mexican Population

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ABSTRACT: We conducted a randomized, crossover study in 23 healthy young female volunteers to compare the bioavailability of two brands of meloxicam (7.5 mg) tablets and to obtain pharmacokinetic parameters of this molecule in Mexican population not reported previously. Two tablets (15 mg) were administered as a single dose on 2 treatment days separated by a 1-week washout period. After dosing, serial blood samples were collected for a period of 72 h. Plasma harvested was analyzed for meloxicam by a modified and validated high-performance liquid chromatography (HPLC) method previously reported. Pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, k_e, MRT and $t_{1/2}$ were determined from plasma concentrations of both formulations, resulting in a C_{max} 120% larger than and a T_{max} 65% faster than those reported in other populations. AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were statistically tested for bioequivalence after log transformation data in a non-balanced design, and no significant differences were found either in 90% classical confidence interval (90% CI) or in Schuirmann test ($p<0.05$); thus, we concluded that bioequivalence existed between both formulations. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: meloxicam; bioequivalence; pharmacokinetics; high-performance liquid chromatography (HPLC)

Introduction

Meloxicam (MXC) (CAS 71125-38-7) (4-hydroxy-2-methyl-*N*-[5-methyl-2-thiazolyl]-2H-1,2,-benzothiazine-3-carboxamide-1,1-dioxide) is a non-steroidal, anti-inflammatory drug (NSAID) that blunts prostaglandin synthesis selectively via type-2 cyclooxygenase (COX-2) inhibition, resulting in inflammation relief [1]. Thus, although the Food and Drug Administration (FDA) recognizes the use of MXC in osteoarthritis and rheumatoid arthritis, its use in extra-articular diseases has been extended [2].

Oral or rectal doses of MXC are well-absorbed with absolute bioavailability of 90% [3]. There were no differences in bioavailability when MXC was administered under fasting conditions or following food intake; maximum plasma concentrations (C_{max}) fluctuated from 1.5 to 1.7 μg/ml and reached 9–11 h (T_{max}) after 30 mg was given orally [4]. MXC is bound to serum albumin at a high level (>99%) and readily penetrates into perivascular spaces, showing an apparent volume of distribution between 0.1 and 0.2 L/kg [5]. MXC is extensively metabolized in liver to four physiologically inactive metabolites that are excreted in both urine and faeces. CYP2C9 plays a major role in oxidative metabolism of MXC,

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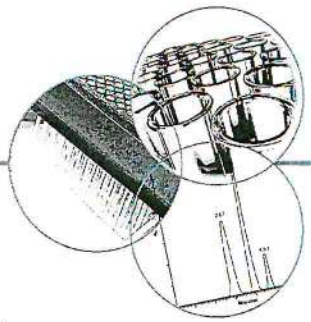

Development of a method by UPLC–MS/MS for the quantification of tizoxanide in human plasma and its pharmacokinetic application

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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Study of the Photodegradation Process of Vitamin E Acetate by Optical Absorption, Fluorescence, and Thermal Lens Spectroscopy

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Abstract The stability of vitamin E acetate exposed to ultraviolet (UV) light was studied using three spectroscopic methods. An ethanol solution of vitamin E acetate was treated with either UVC light (254 nm) or UVA light (366 nm) during a period of 10 min followed by a study of UV–Vis optical absorption, then by fluorescence spectroscopy excitation by UV radiation at either 290 nm or 368 nm and, finally the solution was studied by thermal lens spectroscopy. Immediately, the same solution of vitamin E acetate was subjected to the UV irradiation process until completion of six periods of irradiation and measurements. UVC light treatment induced the appearance of a broad absorption band in the range of 310 nm to 440 nm with maximum absorbance at 368 nm, which progressively grew as the time of the exposure to UVC light increases. In contrast, UVA light treatment did not affect the absorption spectra of vitamin E acetate. Fluorescence spectra of the vitamin E acetate (without UV light treatment) showed no fluorescence when excited with 368 nm while exciting with 290 nm, an intense and broad emission band (300 nm to 440 nm) with a maximum at

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Development of an Ultra-performance Liquid Chromatography Technique Coupled with Mass Spectrometry for the Measurement of Tacrolimus in Micro-samples of Whole Blood, and its Application on a Pharmacokinetic Trial

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Abstract

Objective: The aim was to develop a rapid, specific, sensitive and accurate chromatographic technique coupled with mass spectrometry for the measurement of tacrolimus (CAS 104987-11-3) in micro-samples of whole blood, and its application on a pharmacokinetic pilot trial.
Methods: A fast gradient was designed in an ultra-performance liquid chromatography, and coupled with a mass spectrometer for the quantification of tacrolimus in 100 µl samples of EDTA whole blood. Multiple reaction monitoring was used for the measurement of tacrolimus (m/z^{+1} 821.49→768.35 Th) and sirolimus as internal standard (m/z^{+1} 931.69→864.39 Th). The method was validated according to Mexican regulatory guidelines. Twenty-four young healthy male volunteers with similar hematocrit values participated in the pharmacokinetic trial; an oral single dose of one 5 mg tacrolimus capsule was administered and kinetic profiles were described since 0 h until 24 h post-dose.
Results: Method showed to be accurate, precise and linear over the range from 1 to 80 ng/ml, having an absolute recovery of 94 %. Molecule was stable for two months at –70 °C, and heparin interfered with its quantification. Total run-time is around 1.5 min. Mean maximum blood concentration was 32.63 ± 1.74 ng/ml, and was reached at 1 h post-dose; elimination half-life was 14.18 ± 5.71 h.
Conclusions: Method developed is not time-consuming, inexpensive, and sensitive enough for its application during pharmacokinetic trials, and can be suitable for therapeutic drug monitoring in transplanted patients. Pharmacokinetic data obtained in Mexican population are quite similar to previously reported in international literature.

Key words

- CAS 104987-11-3
- Immunosuppressive agents
- Mass spectrometry
- Tacrolimus, measurement in whole blood, pharmacokinetics
- Ultra performance liquid chromatography

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