

In vitro / in vivo correlation of fast release mephenamic acid microspheres in humans

Aly H. Nada

***Departments of Pharmaceutics
Faculty of Pharmacy
Kuwait University
Kuwait***





Outline

- Introduction & objectives
- Summary of formulation of fast-release mephenamic acid (MFA).
- Assess the bioavailability of an optimized MFA microspheres (test) against Ponstan[®] capsule (reference) in healthy volunteers:
 - Study design
 - Method of analysis
 - Calculation of PK parameters (C_{max} , T_{max} , AUC_{0-12} and $AUC_{0-\infty}$)
 - Relative bioavailability
- Relevant in-vitro parameter; 90%-dissolution (T90)
- In-vitro/in-vivo correlation
- Conclusions

Introduction

- Mephenamic acid has been widely used analgesic and anti-inflammatory agent.
- MFA is characterized by low aqueous solubility and limited dissolution rate and bioavailability.
- In a previous work, we have reported on the formulation of MFA microspheres by emulsion congealing method, and employing the ammonium salt of the drug to enhance the rate of dissolution.

Etman M, et al., J Microencapsul, 2010; 27:640-656

Objectives

- Assess the bioavailability of MFA from the selected microsphere formulation (Formula O) in healthy male volunteers, Establish an *in vitro/in vivo* correlation.
- Compare the performance of the test microspheres against a commercial conventional MFA capsule available on the local market as a reference product (Ponstan[®]).
- Establishment of a useful in-vitro/in-vivo correlation.

Methods

Preparation of MFA Microspheres

- MFA ammonium salt was first prepared by adding MFA to molten PEG and 5 ml of 5% ammonium hydroxide solution was then added drop-wise while stirring, forming a transparent melt which was then dried overnight in a desiccator.
- The microspheres were subsequently prepared using hot emulsion congealing technique.
- The aqueous phase (PEG and ammonium salt of MFA) and the oily phase (50 ml liquid paraffin containing 0.5% w/v Span 80) were heated to 70°C prior to emulsification to form W/O emulsion.

- Hardening of the aqueous internal phase and the formation of the microspheres were accomplished by pouring equal volume of ice cold liquid paraffin (4°C) into the beaker containing the hot emulsion.
- The congealed microspheres were separated from the oily phase by filtration with decantation, followed by washing with cyclohexane, drying at room temperature and sieving.

- Ammonium salt of mephenamic acid exhibited a higher rate of dissolution. The described method for microsphere preparation was simple, efficient and resulted in spherical and stable microspheres with enhanced drug release.
- Optimum conditions for preparing fast-release microspheres include:
The use of moderate molecular weight PEG, inclusion of Span and Tween, increasing the ratio of the water soluble polymer to drug in the prepared microspheres, use of mineral oil and application of a high speed of stirring (2000 rpm) during emulsification.

Bioavailability Study

Subjects, Products and Design

- Four healthy volunteers, 27 to 36 years and 60 to 90 kg, participated in the study.
- The health state of was ascertained by biochemical blood analysis before the study.
- The subjects did not administer other medication for one week period before initiation of the study and throughout the study period.
- Test product: MFA microspheres (Formula O = 250 mg MFA) filled in hard gelatin capsules
- Reference: Ponstan[®] capsule (250 mg MFA)

- The study followed a random crossover design, separated by one week washout period.
- The subjects were given either product along with 250 ml of water following an overnight fasting, which was continued for four hours after dose administration, followed by a standard meal Venous blood samples (5ml) were collected at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after drug administration.
- The heparinized blood samples were used to separate plasma fractions by centrifugation and frozen at -20 °C pending analysis.

HPLC Analysis of Plasma Samples

- HPLC with ultraviolet detection, using reverse phase column (μ Bondapack C18, 10 μ m, 3.9 x 300 mm Waters).
- Five μ l of an internal standard solution (Etodolac 50 μ g/ml in the mobile phase) was used and precipitation of plasma proteins was accomplished by 0.5 ml acetonitrile.
- The mixture was then shaken and centrifuged for and the clear supernatant solution (20 μ l) was then injected directly into the HPLC column.

- Standards were prepared by adding aliquots of MFA standard solutions to 0.2 ml blank plasma to cover a concentration range from 5 to 25 μg/ml.
- The mobile phase consisted of CH₃CN : H₂O (1:1 v/v) adjusted to pH 4.0 with glacial acetic acid. The flow rate was 1 ml/min, and the eluents were detected at 285 nm.
- Under such conditions, the retention time for MFA and Etodolac (internal standard) were 10 and 6 minutes, respectively.

RESULTS

- Validation parameters of the assay were confirmed by calculation of interday precision (SD 0.60-1.29) and linearity (“r” values very close to unity, 0.987 to 0.999).
- The mean plasma concentrations vs. time (Fig. 1) were fitted to a stripping computer program and the generated relevant pharmacokinetic parameters are summarized in Table 1.
- The mean C_{\max} from the test product was one and half time more than the reference; namely 5.91 ± 0.60 and $3.58 \pm 0.67 \mu\text{g/ml}$, respectively.

Table 1: Pharmacokinetic parameters for the test and reference products

Pharmacokinetic Parameters	Test (\pm S.D.)	Reference (\pm S.D.)
AUC₀₋₁₂, ug.h/ml	35.553 (4.38)	20.618 (7.412)
AUC_{0-∞}, ug.h/ml	38.028 (2.276)	22.393 (10.113)
C max, ug/ml	5.914 (0.604)	3.575 (0.671)
T max, h	1.873 (0.482)	2.143 (0.20)
K_{el}, hr⁻¹	0.2209 (0.056)	0.343 (0.127)
Elimination t_{1/2}, hr	3.46 (0.758)	2.419 (1.139)
K_{ab}, h⁻¹	2.1414 (1.852)	0.9596 (0.208)
Absorption t_{1/2}, hr	0.5347 (0.258)	0.6439 (0.208)
MRT, h	5.715 (0.965)	5.057 (1.215)
Lag Time, h	0.188 (0.121)	0.268 (0.178)

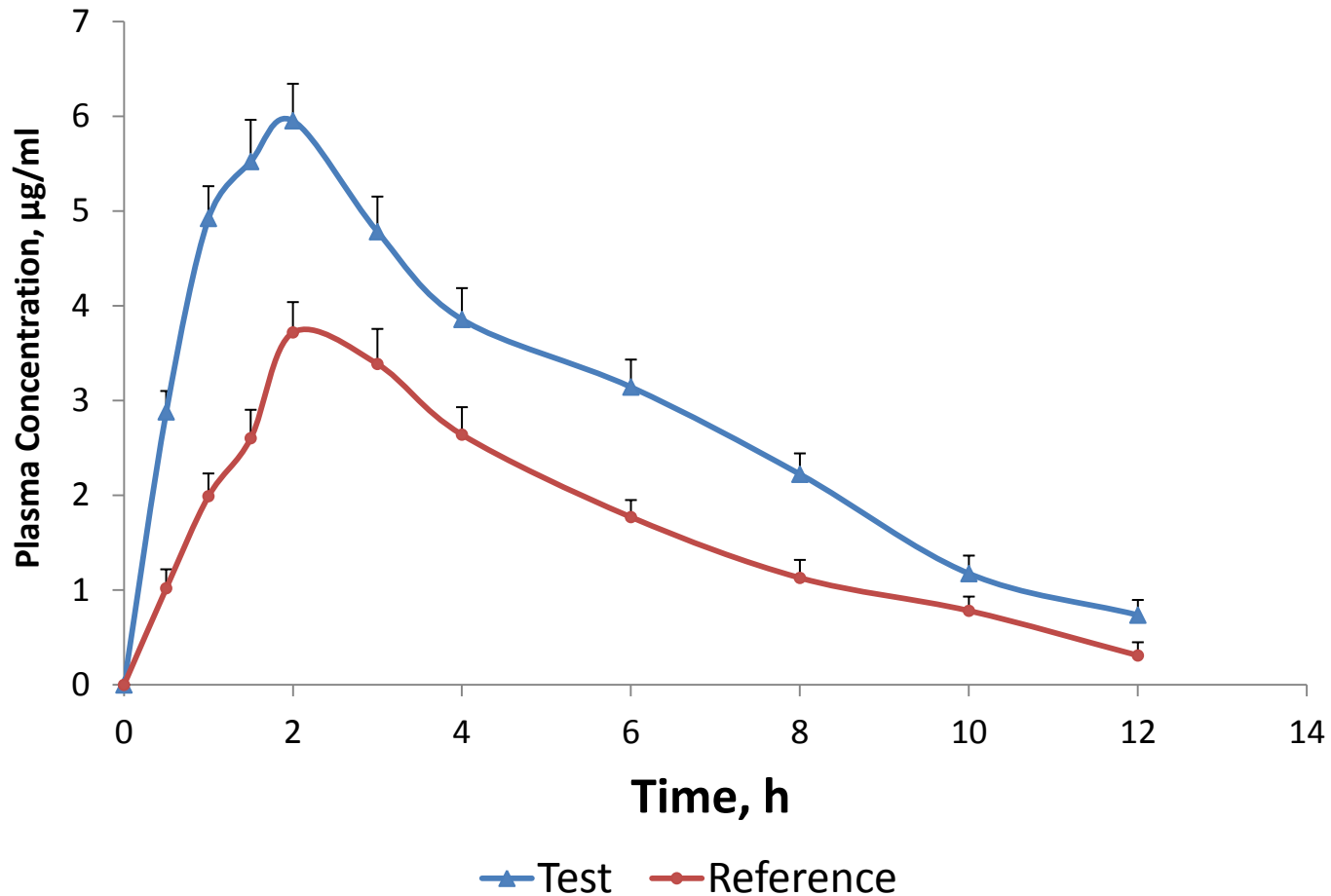


Fig. 1: Mean plasma mephenamic acid (MFA) concentration following oral administration of fast release microspheres of Formula O (Test) and Ponstan[®] capsule (Reference), each containing 250 mg MFA.

- The fast-release formulation showed significantly higher mean plasma levels compared with the conventional reference MFA capsules (Fig. 1); as indicated by shorter mean T_{\max} ($1.87\text{h} \pm 0.48$), compared with the reference product ($T_{\max} = 2.14\text{h} \pm 0.02$) (Table 1).
- In addition, the test formulation improved the bioavailability of the drug leading to about 1.7 fold increase in AUC, 1.6 fold increase in C_{\max} and a decrease in T_{\max} (0.8 fold) relative to the reference product (Table 1).

In vitro / in vivo correlation

- Good correlations were found for the relationships between the time to 90 %-dissolution of MFA (T₉₀) of the test formulation and each of AUC₀₋₁₂ ($y = -39.65 + 4.03 x$, $r = 0.994$) and T_{max} ($y = -5.79 + 0.42x$, $r = 0.994$) (Fig. 2).
- Furthermore, good correlation between the %-drug released and the corresponding plasma concentrations of the tested MFA microcapsules ($Y = 0.085X - 4.742$, $r = 0.9664$) was observed.

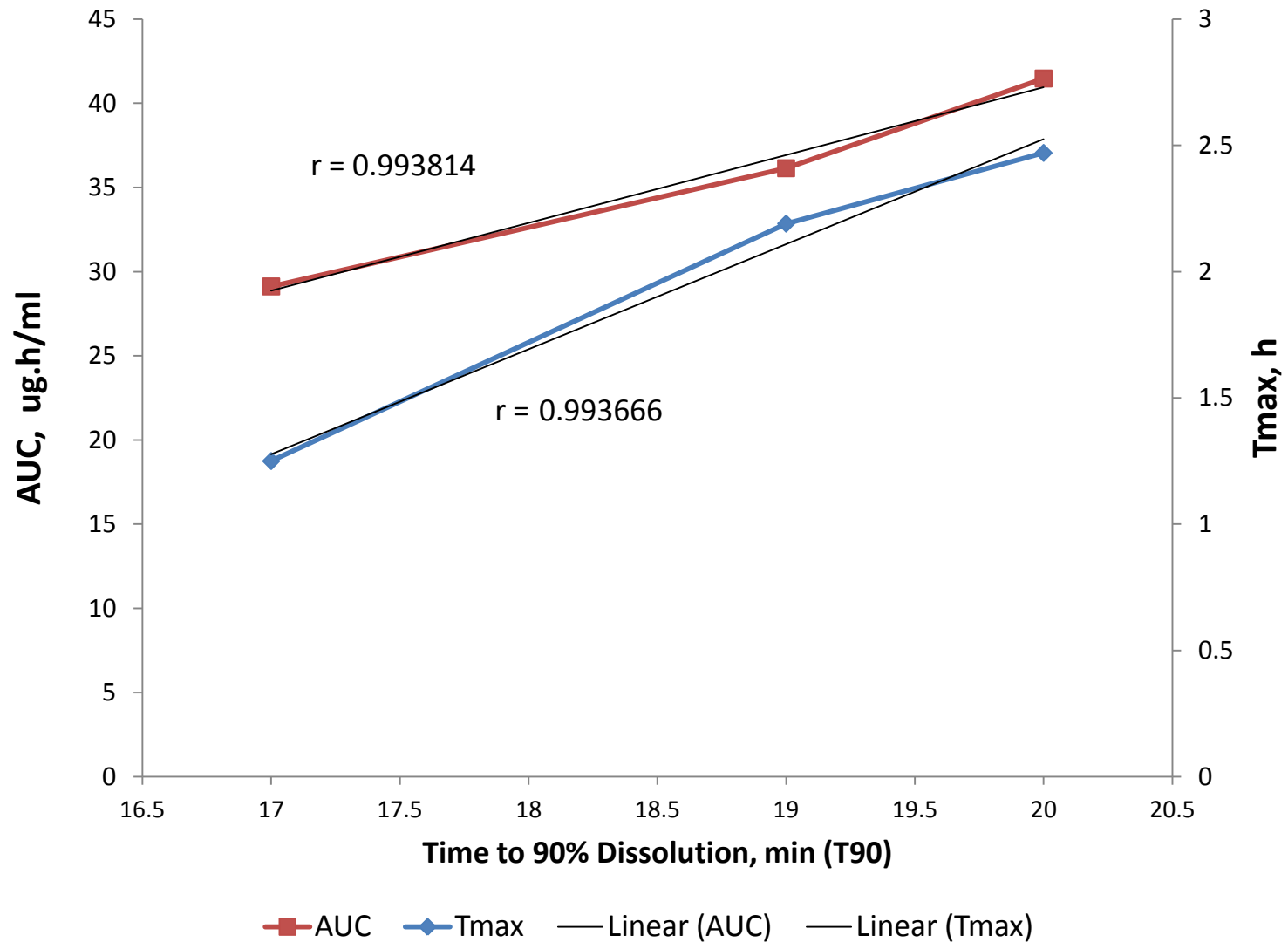


Fig. 2: Correlation of the time to 90% mephenamic acid (MFA) dissolution (T90) and the corresponding individual values of each of AUC0-12 and Tmax

- 1. Mohamed A. Etman^a**
- 2. Abdel-Azim R. Ebian^a**
- 3. Ragwa M. Farid^b**

Departments of Pharmaceutics,

*^aFaculty of Pharmacy, Alexandria University, Alexandria,
Egypt*

*^bFaculty of Pharmacy & Drug Manufacturing, Pharos
University, Alexandria, Egypt ad*



Lighthouse of Alexandria



Citadel of Qaitbay



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Thank You!

The image features the words "Thank You!" in a highly decorative, hand-drawn style. The letters are thick and filled with various colors and patterns. The 'T' is yellow with a red vertical line and a wavy border. The 'h' is orange with a red wavy border. The 'a' is green with a black dotted border. The 'n' is purple with a black dotted border. The 'k' is orange with a red wavy border. The 'Y' is green with a black dotted border. The 'o' is pink with a black dotted border. The 'u' is blue with a black dotted border. The '!' is blue with a green circle. There are several stylized flowers: a blue flower with a purple center, a pink flower with a yellow center, and a purple flower with a blue center. The background is white.