# Prediction of Oral Absorption: 6-Fluoroquinolones

*Mª del Val Bermejo Sanz, Ph.D.*
*Depto. Farmacia y Tecnología Farmacéutica*
*Facultad de Farmacia. Universidad de Valencia*
*Valencia, España*

*Strategies for Oral Drug Delivery*
*Oral Absorption Regulation and Evaluation*
*March 11-16, 2001 Garmisch Partenkirchen, Germany*



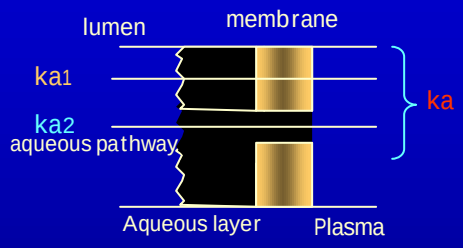
## *Prediction of Oral Absorption: 6-Fluoroquinolones*

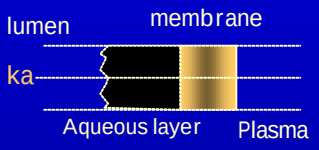
"Bioavailability prediction in drug development: fluoroquinolones." CICYT (SAF 96-1710)

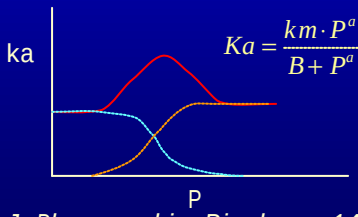
Director: Prof. JM Plá Delfina

- Absorption-partition relationships
- Bioavailability predictions

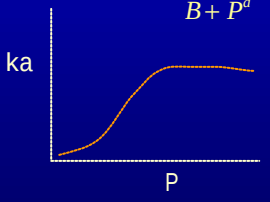
# Absorption-Partition Correlations




$$Ka = \frac{km \cdot P^a}{B + P^a} + \frac{kp \cdot C}{C + P^d}$$

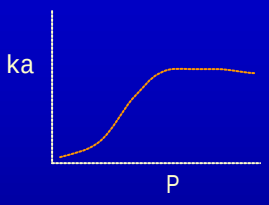


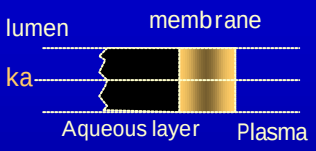

$$Ka = \frac{km \cdot P^a}{B + P^a}$$

*J. Pharmacokin. Biopharm. 14(6),615-33 (1986)*
*J. Pharmacokin. Biopharm. 15(6),633-43 (1987)*



# Absorption-Partition Correlations




$$\frac{1}{ka} = \frac{1}{kaq} + \frac{1}{kmem}$$

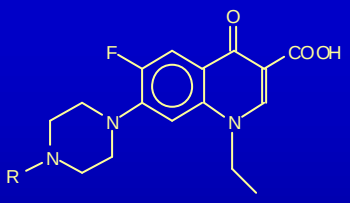
Higuchi- Ho

$$Ka = \frac{C \cdot P^d}{1 + E \cdot \sqrt{M} \cdot P^d}$$

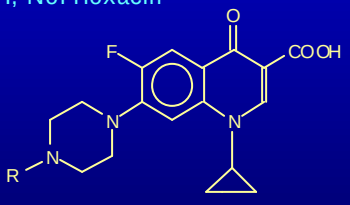
- Correlations in Colon or
- in Small intestine with Compounds MW>250

*Journal of Pharmaceutical Sciences* **84,** 777-782 (1995).

# Absorption-Partition Correlations



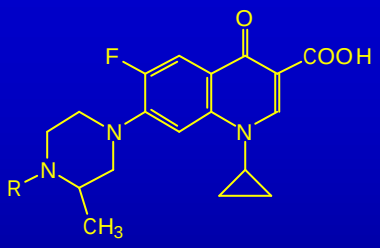
R= H, Norfloxacin



R= H, Cyprofloxacin

$R= CH_3$ , $CH_3-(CH_2)_n-$ n=1-5

Homologous Compounds

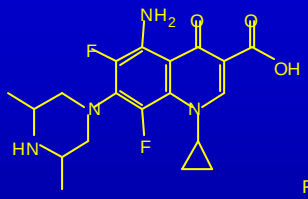


| R | Name |
|---|---|
| H | CNV 97100 |
| $-CH_3$ | CNV 97101 |
| $-CH_2-CH_3$ | CNV 97102 |
| $-(CH_2)_2-CH_3$ | CNV 97103 |
| $-(CH_2)_3-CH_3$ | CNV 97104 |

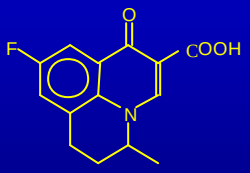
*CENAVISA S.A. Spain*



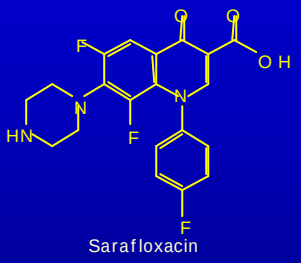
# Absorption-Partition Correlations



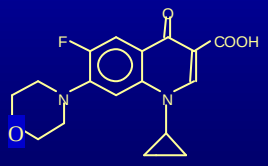
Sparfloxacin



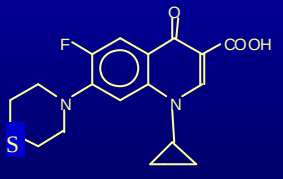
Flumequine



Sarafloxacin

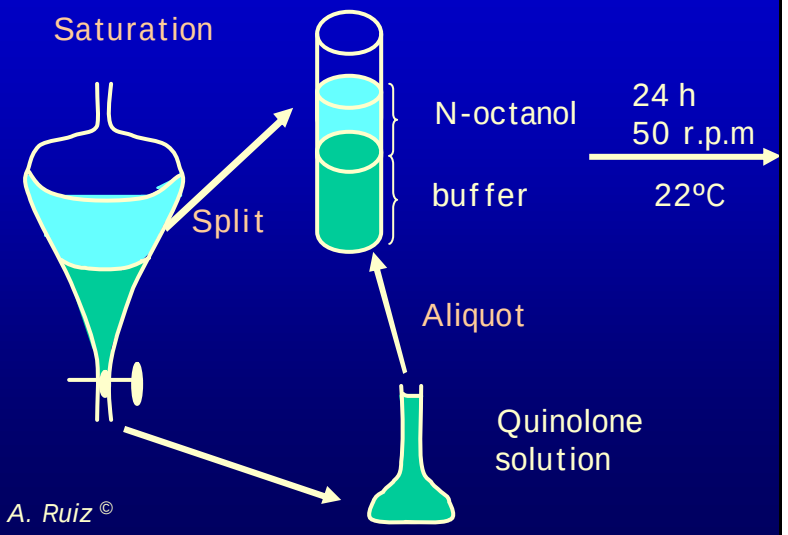


CENAVISA 8804



CENAVISA 8919

# Absorption-Partition Correlations






# Absorption-Partition Correlations

Partition Coefficient:

Organic Phase: n-Octanol

Aqueous Phase: aqueous buffer solution at pH. 7.00

Phase Volume adjusted for each compound

- Saturation
- Dissolution of quinolone in aqueous phase
- Equilibrating during 24 h at 22ºC
- Analysis of the aqueous concentration by HPLC

$$P = \frac{C_o}{C_a} = \frac{(Q_{ai} - Q_{af})/V_o}{Q_{af}/V_a}$$

# Absorption-Partition Correlations

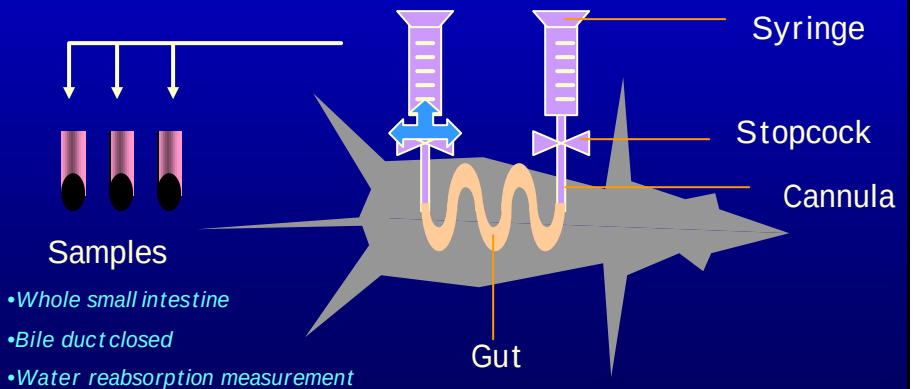
Absorption studies:

- Solutions of compounds on saline buffered at pH. 7.00
- Concentration of each compound far enough of its Cs
- Perfused Volume: 10 mL



# Absorption-Partition Correlations

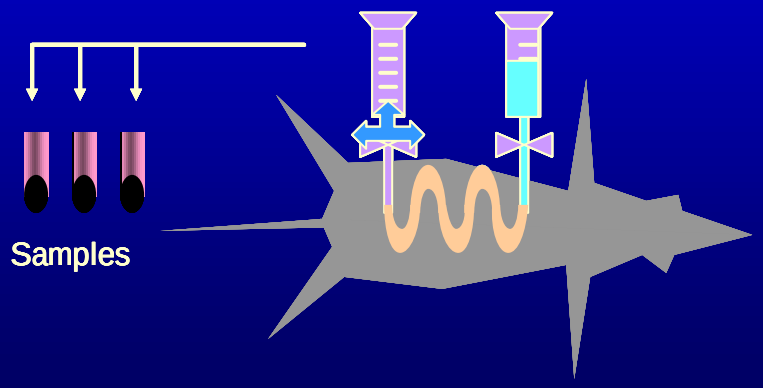
Closed Loop Perfussion Technique: Doluisio's Method




- *Whole small intestine*
- *Bile duct closed*
- *Water reabsorption measurement*

## Absorption-Partition Correlations

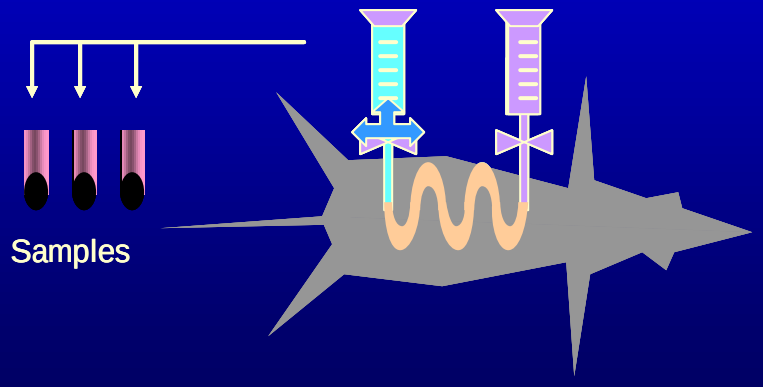
Closed Loop Perfussion Technique: Doluisio's Method





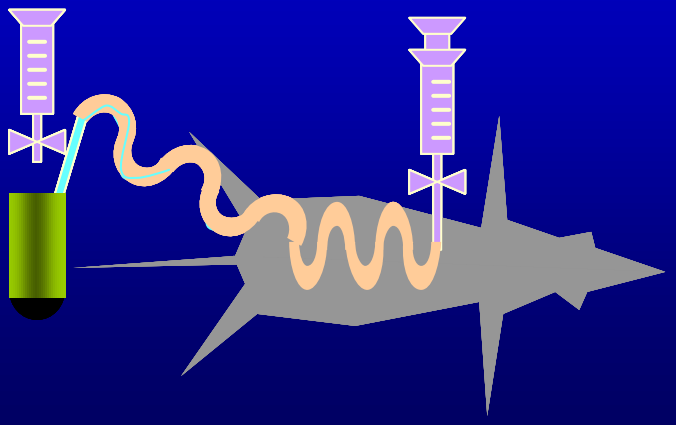

## Absorption-Partition Correlations

Closed Loop Perfussion Technique: Doluisio's Method



# Absorption-Partition Correlations

Closed Loop Perfussion Technique: Doluisio's Method





# Absorption-Partition Correlations

*Water reabsorption correction*

$$V_t = V_0 - k \cdot t$$

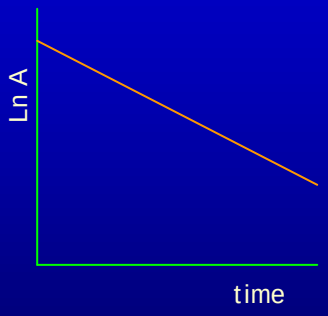
$$A_c = A_{\exp} \cdot \frac{V_t}{V_0}$$

# Absorption-Partition Correlations

Absorption rate constant:

$$A = A_0 \cdot e^{-ka \cdot t}$$

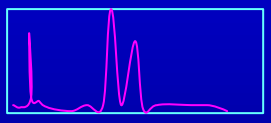
ka, mean value of six animals



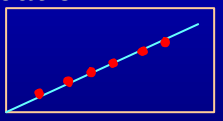



# Absorption-Partition Correlations

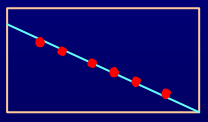
Analysis: HPLC fluorimetric detection



Method validation

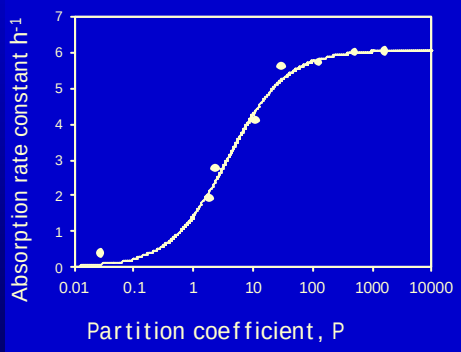


Stability of compound in luminal fluid

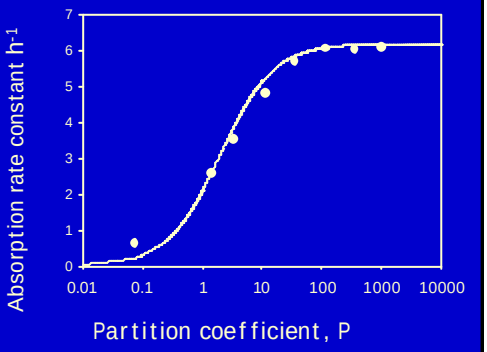


# Absorption-Partition Correlations

Norfloxacin homologous




Cyprofloxacin homologous

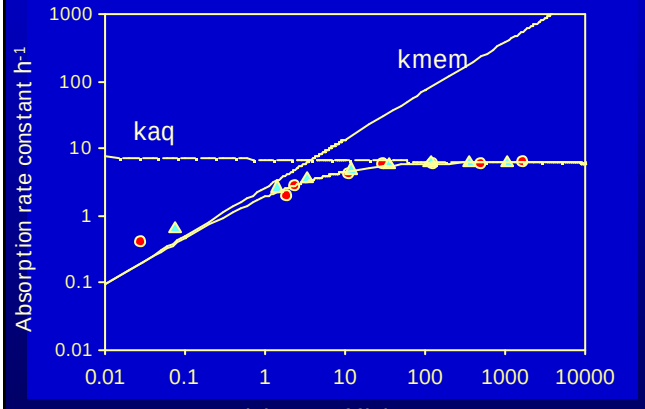



*Journal of Pharmaceutical Sciences* **84,** 777-782 (1995).



# Absorption-Partition Correlations

$$\frac{1}{ka} = \frac{1}{kaq} + \frac{1}{kmem}$$




| Kaq ($h^{-1}$) | Kmem ($h^{-1}$) |
|---|---|
| 7.073 | 0.195 |
| 6.944 | 0.397 |
| 6.923 | 3.322 |
| 6.801 | 4.070 |
| 6.782 | 4.795 |
| 6.667 | 6.210 |
| 6.649 | 14.813 |
| 6.541 | 15.359 |
| 6.524 | 30.267 |
| 6.422 | 33.761 |
| 6.405 | 79.701 |
| 6.309 | 85.387 |
| 6.293 | 181.075 |
| 6.201 | 233.991 |
| 6.186 | 389.164 |
| 6.099 | 545.016 |

# Absorption-Partition Correlations

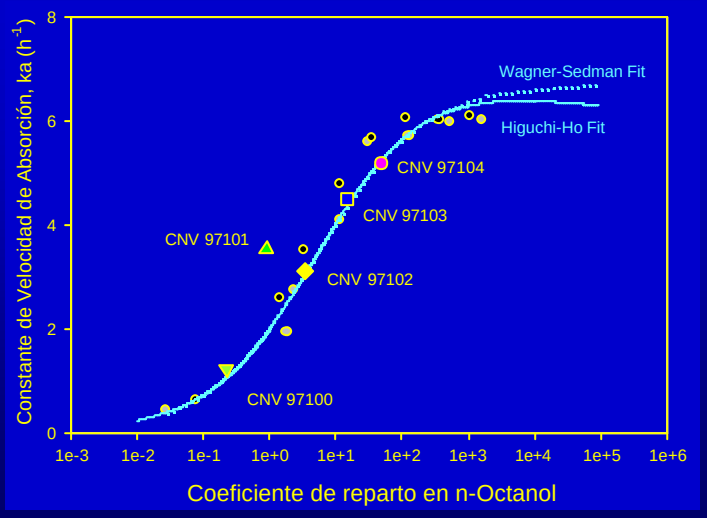
| Compound | $MIC_{90}$ | Compound | $MIC_{90}$ |
|---|---|---|---|
| Norfloxacin | 0.1 | Ciprofloxacin | 0.025 |
| N'-Methylnorfloxacin | 0.1 | N'-Methylciprofloxacin | 0.025 |
| N'-Ethylnorfloxacin | 0.2 | N'-Ethylciprofloxacin | 0.05 |
| N'-Propylnorfloxacin | 0.4 | N'-Propylciprofloxacin | 0.1 |
| N'-Butylnorfloxacin | 0.4 | N'-Butylciprofloxacin | 0.2 |
| N'-Pentylnorfloxacin | 0.4 | N'-Pentylciprofloxacin | 0.2 |
| N'-Hexylnorfloxacin | 1.6 | N'-Hexylciprofloxacin | 0.4 |
| N'-Heptylnorfloxacin | 3.2 | N'-Heptylciprofloxacin | 0.4 |

$MIC_{90}$ of the compounds against *E. Coli* ATCC 25922

*Journal of Pharmaceutical Sciences* **84,** 777-782 (1995).



# Absorption-Partition Correlations




*Ana Ruiz-García. Ph.D Dissertation. University of Valencia. November 2000*

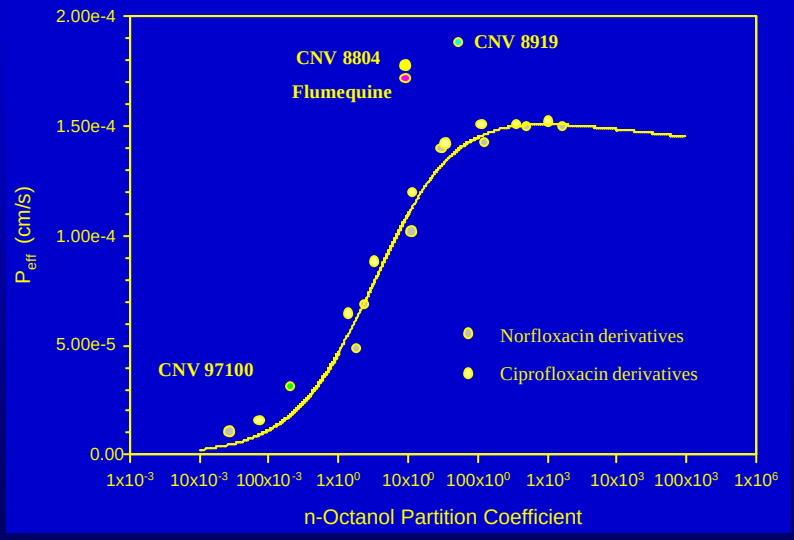
# Absorption-Partition Correlations

| Quinolone | ka-pred HH | ka exp. | ER% |
|---|---|---|---|
| 97100 | 1.06 | 1.24 | -14.9 |
| 97101 | 1.91 | 3.54 | -46.0 |
| 97102 | 3.06 | 3.12 | -2.1 |
| 97103 | 4.35 | 4.5 | -3.2 |
| 97104 | 5.20 | 5.16 | 0.8 |

*Ana Ruiz-García. Ph.D Dissertation. University of Valencia. November 2000*



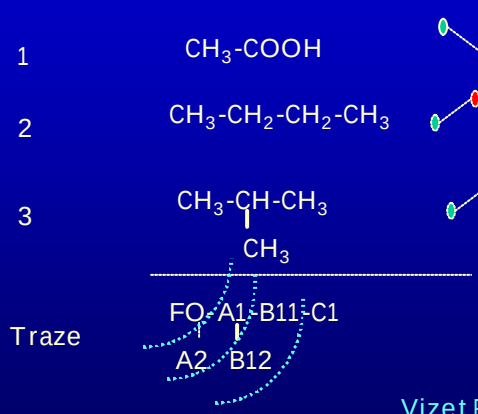
# Absorption-Partition Correlations




*Journal of Pharmaceutical Sciences* (4) 398-405, 1998.

# Absorption-Partition Correlations : QSAR methodologies in drug absorption

Instituto de Topología y Dinámica de Sistemas ITODYS.
Université Paris VII. France



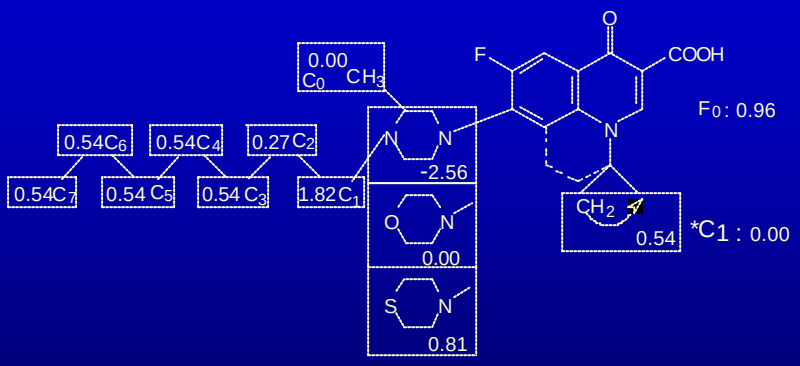

| | Vector | A1 | A2 | B11 | B12 | C1 |
|---|---|---|---|---|---|---|
| 1 | | 0 | 1 | 0 | 0 | 0 |
| 2 | | 1 | 0 | 1 | 0 | 1 |
| 3 | | 1 | 0 | 1 | 1 | 0 |

Vizet P. PATQSAR©. Version 2.55. 1997

*Journal of Pharmaceutical Sciences* (4) 398-405, 1998.



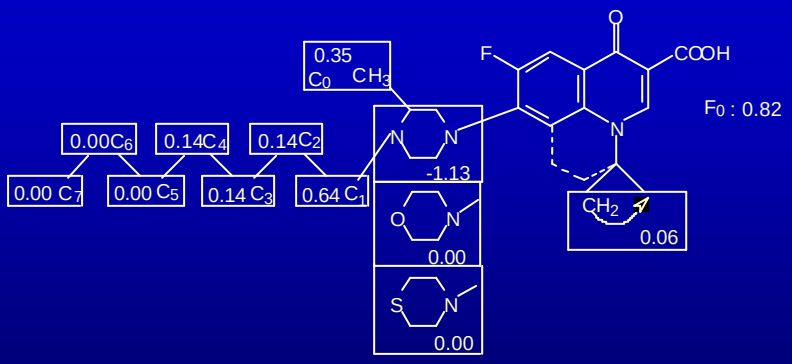
# Absorption-Partition Correlations : QSAR methodologies in drug absorption




Lipophilicity-structure correlation

*Journal of Pharmaceutical Sciences* (4) 398-405, 1998.

# Absorption-Partition Correlations : QSAR methodologies in drug absorption

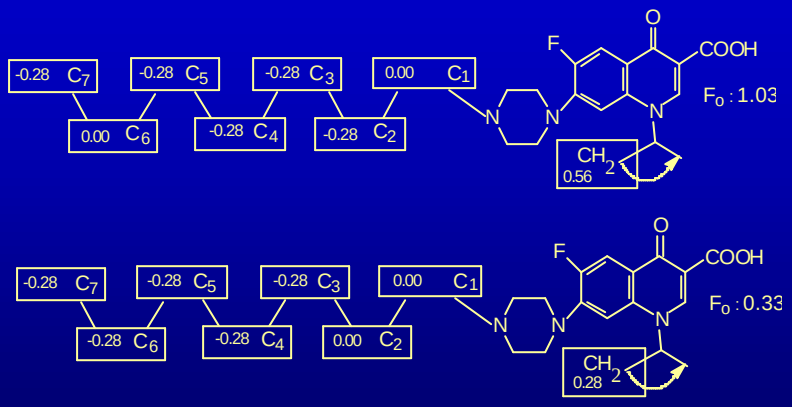



Absorption-structure correlation

*Journal of Pharmaceutical Sciences* (4) 398-405, 1998.



# Absorption-Partition Correlations : QSAR methodologies in drug absorption

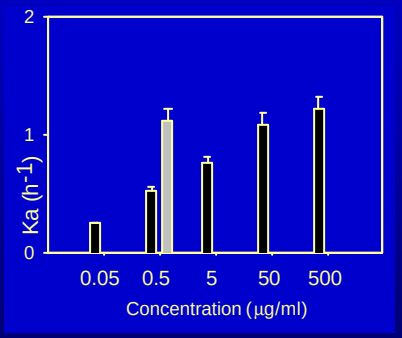



Structure- antibacterial activity correlation

*Journal of Pharmaceutical Sciences* (4) 398-405, 1998.

# Absorption-Partition Correlations

CNV 97 100



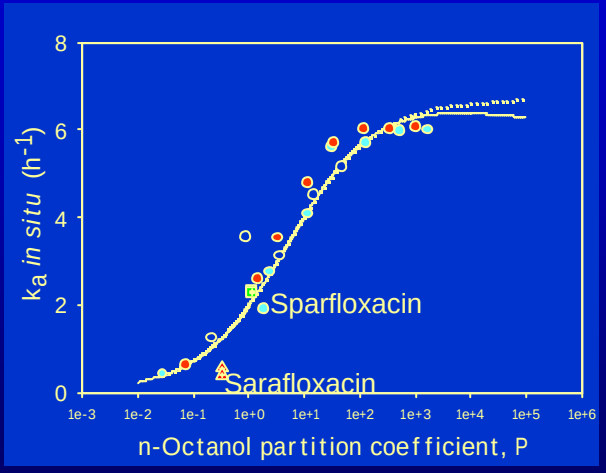

| Conc.(mg/mL) | $k_a(h^{-1})$ | DE |
|---|---|---|
| 500 | 1.215 | 0.107 |
| 50 | 1.081 | 0.110 |
| 5 | 0.757 | 0.050 |
| 0.5 | 0.523 | 0.040 |
| 0.05 | 0.242 | 0.010 |
| 0.5+V | 1.114 | 0.113 |

| Conc (µg/mL) | 500 | 50 | 5 | 0.5 | 0.05 | 0.5+V |
|---|---|---|---|---|---|---|
| 500 | - | - | - | - | - | - |
| 50 | NS | - | - | - | - | - |
| 5 | S | S | - | - | - | - |
| 0.5 | S | S | S | - | - | - |
| 0.05 | S | S | S | S | - | - |
| 0.5+V | NS | NS | S | S | S | - |

*Gonzalez I. et al.. V SEFIG meeting, Valencia 2001*



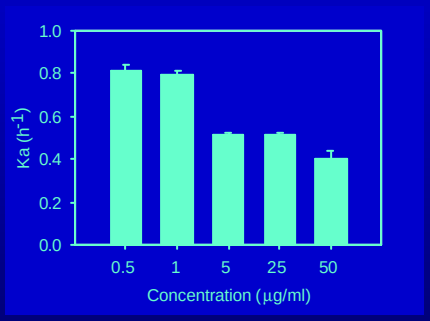
# Absorption-Partition Correlations




*Fernández C. et al. V SEFIG meeting, Valencia 2001*

# Absorption-Partition Correlations

## Sarafloxacin




| *Conc(mg/mL)* | *$k_a(h^{-1})$* | *DE* |
|---|---|---|
| 50 | 0.399 | 0.044 |
| 25 | 0.512 | 0.016 |
| 5 | 0.513 | 0.014 |
| 1 | 0.789 | 0.025 |
| 0.5 | 0.816 | 0.026 |

| Conc (µg/mL) | 50 | 25 | 5 | 1 | 0.5 |
|---|---|---|---|---|---|
| 50 | - | - | - | - | - |
| 25 | S | - | - | - | - |
| 5 | S | NS | - | - | - |
| 1 | S | S | S | - | - |
| 0.5 | S | S | S | NS | - |

*Fernández C. et al. V SEFIG meeting, Valencia 2001*



# STUDY OF THE INTESTINAL ABSORPTION OF CIPROFLOXACIN IN RATS.

Figure 1

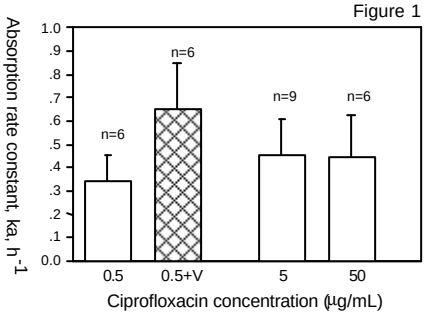
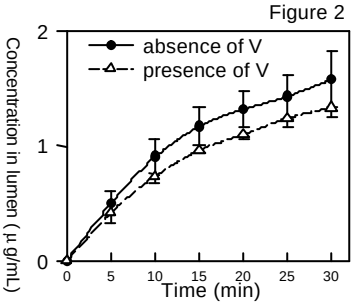



Figure 2




*Rodríguez M et al. 1999 AAPS annual meeting*

## INFLUENCE OF P-GLYCOPROTEIN ON THE INTESTINAL ABSORPTION OF GREPAFLOXACIN.

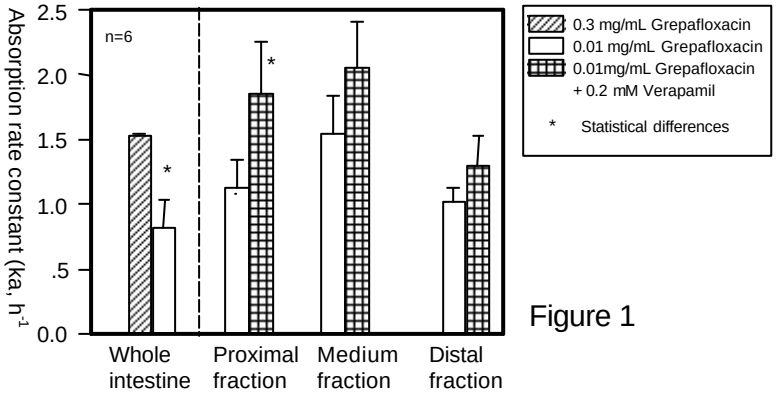



Figure 1

*Sánchez-Castaño G. et al. 1999 AAPS annual meeting*

Grepafloxacin donated by GLAXO



## Bioavailability prediction in drug development

$$F = Fa \cdot (1 - E_g) \cdot (1 - E_h)$$

$E_g$= gut or liver first pass effects

$E_h$= biliary excretion

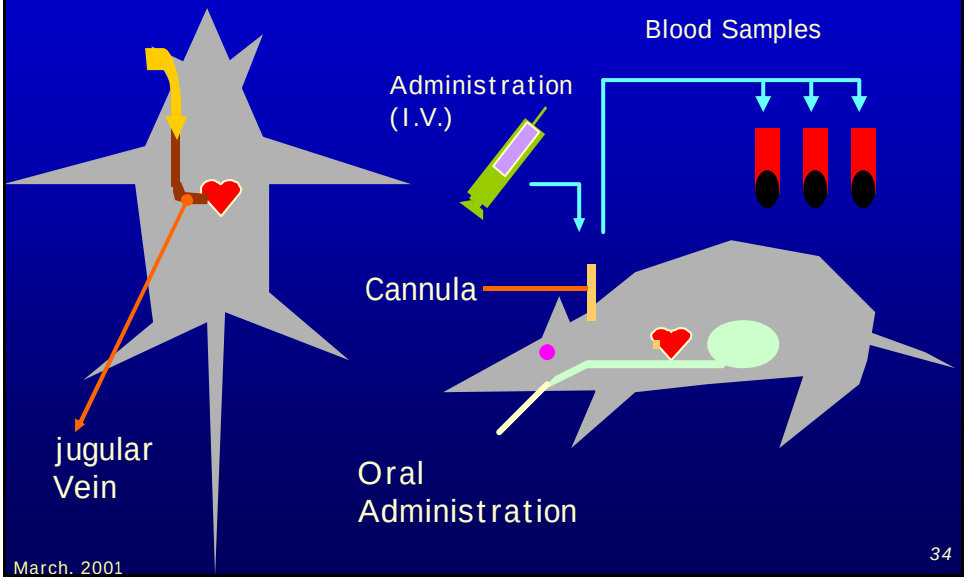
## Bioavailability prediction in drug development

$$F_a = 1 - e^{-ka \cdot T}$$

$$F_a = 1 - e^{-\left(\frac{B \cdot P^a}{1 + C \cdot \sqrt{M} + P^a}\right) T}$$



## Bioavailability prediction in drug development



# Bioavailability prediction in drug development

Oral administration
Group A (8-12 rats)

I.V administration
(bolus or perfusion)
Group B (8-12 rats)

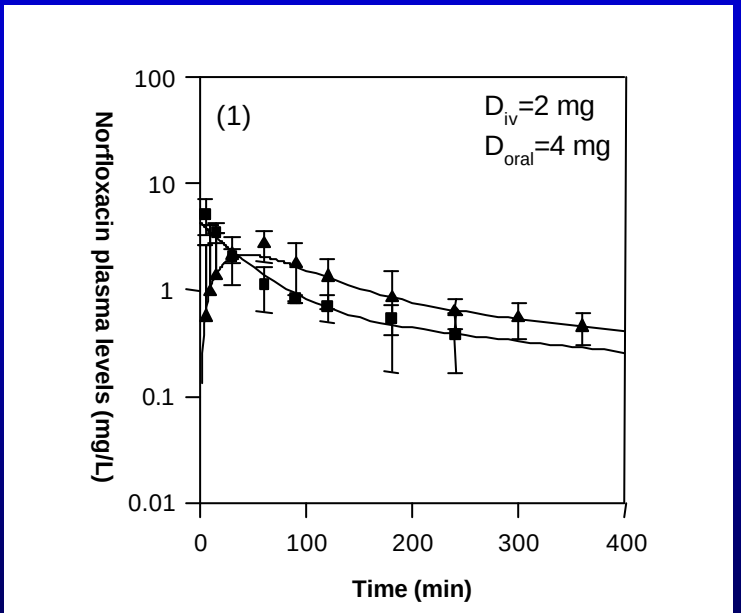
Plasma sampling and Analysis

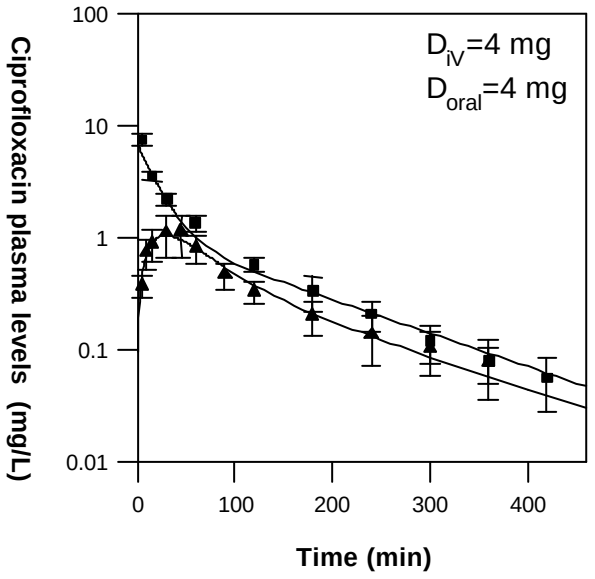
$AUC_0^{\infty}$ calculation

$AUC_0^{t}$ + $AUC_t^{\infty}$

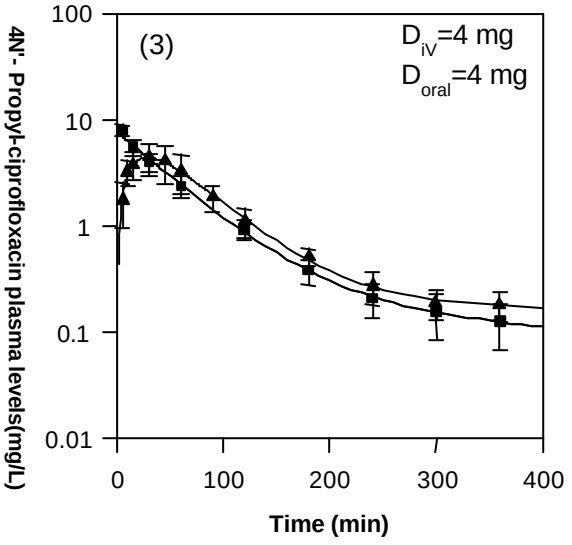
(Trapezoidal rule) ( $C_t/k$)

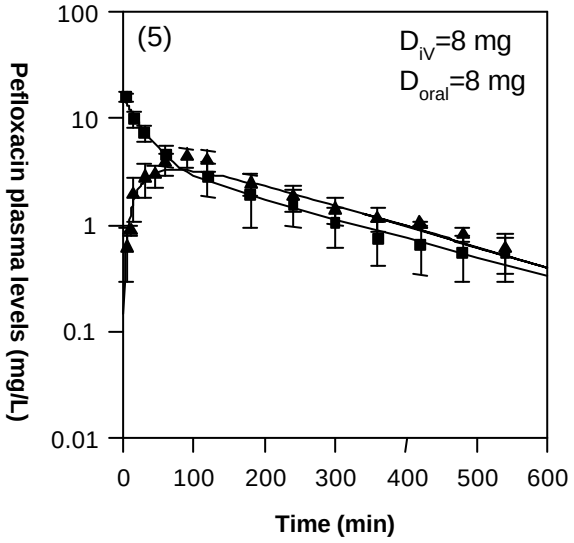




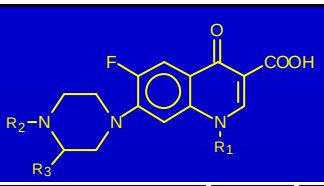

Ciprofloxacin plasma levels (mg/L)
100
10
1
0.1
0.01
0
100
200
300
400
Time (min)
$D_{iV}$=4 mg
$D_{oral}$=4 mg



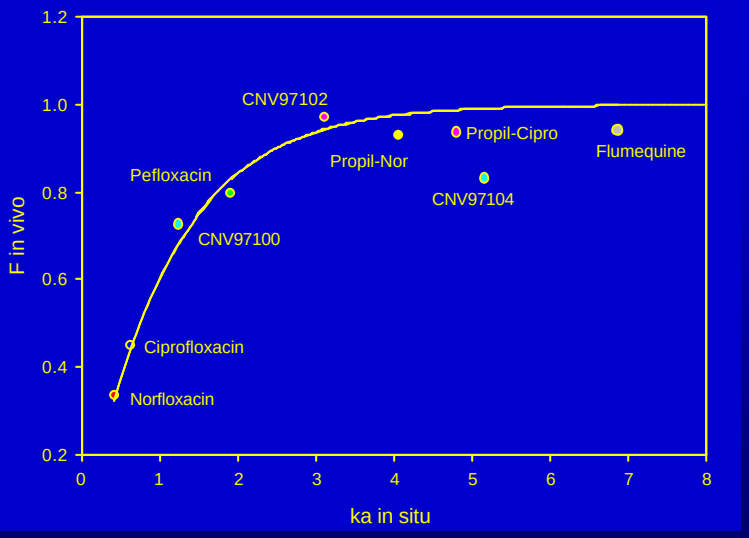

4N'- Propyl-ciprofloxacin plasma levels(mg/L)
(3)
100
10
1
0.1
0.01
0
100
200
300
400
Time (min)
$D_{iV}$=4 mg
$D_{oral}$=4 mg






| Tested quinolone | Substituents in the formula | | | Molecular Weight (M) | P (n-octanol) | $k_a$ ($h^{-1}$) *in situ* |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | |
| Norfloxacin | Ethyl | H | H | 319 | 0.028±0.002 | 0.42±0.07 |
| Pefloxacin | Ethyl | Methyl | H | 333 | 1.883±0.050 | 1.92±0.18 |
| 4N'-Propyl-norfloxacin | Ethyl | Propyl | H | 361 | 11.27±0.27 | 4.07±0.40 |
| Ciprofloxacin | Cyclopropyl | H | H | 331 | 0.075±0.003 | 0.63±0.08 |
| 4N'-Propyl-ciprofloxacin | Cyclopropyl | Propyl | H | 373 | 11.80±0.20 | 4.80±0.52 |
| 3'-Methyl-ciprofloxacin | Cyclopropyl | H | Methyl | 345 | 0.209±0.001 | 1.24±0.07 |
| Flumequine | Occupied by a 1-8 ring | Without 7N-piperazinyl substituent | | 261 | 9.40±0.90 | 6.87±1.09 |



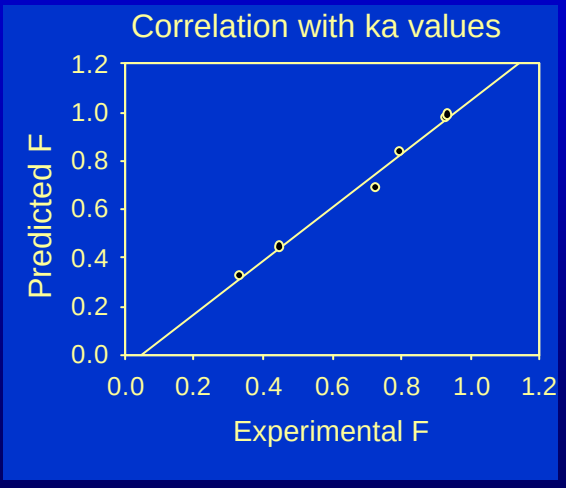

Journal of Pharmaceutical Sciences. 89(11)1395-1403(2000)

European Journal of Biopharmaceutics and Pharmacokinetics. 48,253-258 (1999).

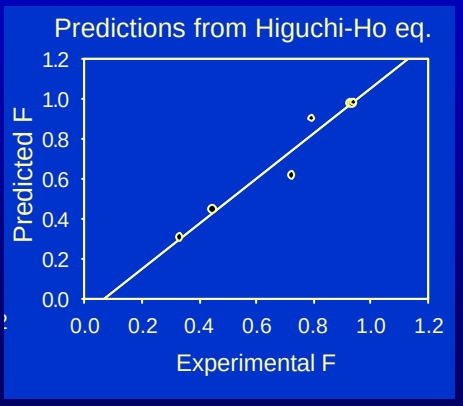
Ana Ruiz-García. Ph.D Dissertation. University of Valencia. November 2000

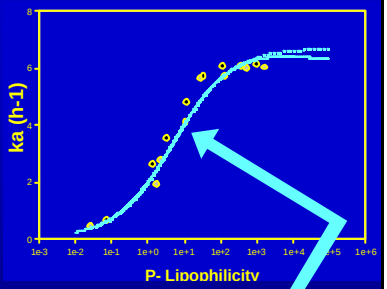


# Bioavailability prediction in drug development



# Bioavailability prediction in drug development




$$F_a = 1 - e^{-\left(\frac{B \cdot P^a}{1 + C \cdot \sqrt{M} + P^a}\right) \cdot T}$$



# Conclusions

1. Lipophilicity seems to be the main factor governing quinolone absorption

2. A Partition coefficient in n-Octanol over 10 produces an absorption constant ~ 2 $h^{-1}$ ⟶ Peff ~ 1* $10^{-4}$ cm/s

3. Quinolones with a in situ permeability over 2*$10^{-4}$ cm/s have a bioavailability aprox. 90%.

## Conclusions

4. The absorption-partition relationship based on Higuchi-Ho equation has a good predictive performance at least with homologous quinolones; for heterologues compounds, the model is still able to predict the partition coefficient for maximum absorption.

5. The absorption rate constant ka determined in situ in rat is a good predictive parameter of oral bioavailability for the studied compounds.

6. The efflux processes observed in situ and in vitro have not a significant influence in vivo in rats at the oral doses used.



## Work in progress

1. In vitro Permeability studies (Caco-2):
   - •Double Log-linear relationship Peff in situ-Peff in vitro.
   - •Efflux process for some quinolones.
2. In situ absorption experiments of 97100 and 97101 at different concentrations: apparent active mechanism?
3. In vivo relevance of 97100 efflux.
4. In vitro metabolism of 97103.

# Research Team

Chairman: José M. Plá-Delfina. Ph. D.

Professors

- Mª del Val Bermejo Sanz. Ph.D.
- Teresa Garrigues Pelufo. Ph.D.
- Virginia Merino Sanjuán. Ph.D.

Teaching assistants

- Gloria Sánchez Castaño Ph.D
- Ana Ruíz García Ph.D

Ph.D candidates

Carlos Fernández

Isabel González

Ricardo Nalda

Margarita Rodríguez