In Vitro Similarity as surrogate for Therapeutic Equivalence

An investigation on the South American Markets

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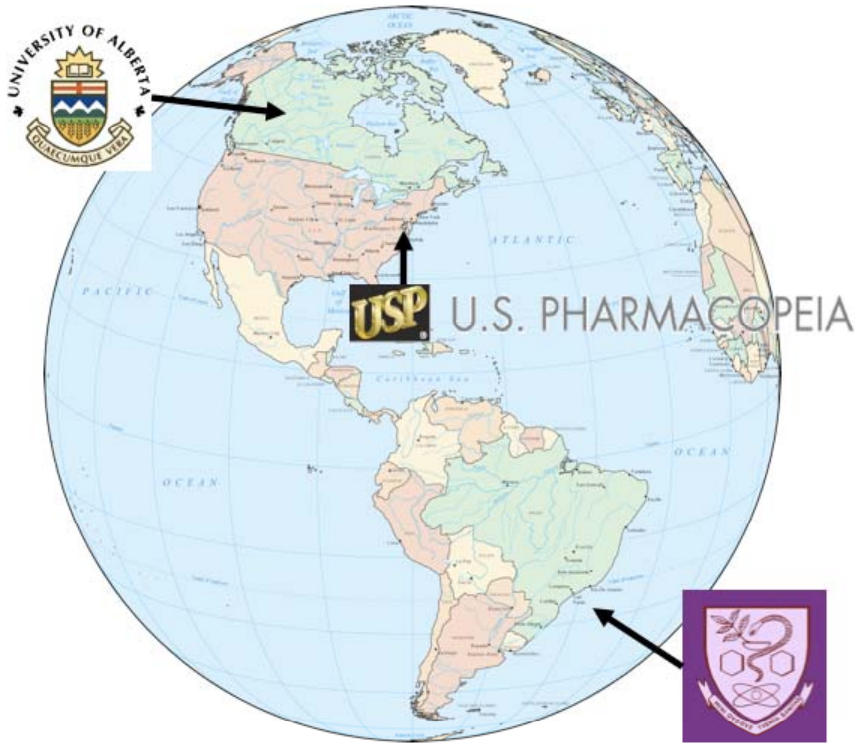


Outline

- **Introduction**
  - WHO Guideline
- **Study Design and Considerations**
  - Zidovudine Results
  - Amoxicillin Results
  - Metronidazole Results
- **Conclusions**

WHO EXPERT COMMITTEE ON SPECIFICATIONS FOR PHARMACEUTICAL PREPARATIONS

The dissolution testing is now emerging as a surrogate equivalence test for certain categories of orally administered pharmaceutical products.

For these products (typically solid oral dosage forms containing APIs with suitable properties) a comparative *in vitro* dissolution profile similarity can be used to document equivalence of a multisource generic with a comparator product.

WHO Guide

On the basis of solubility and permeability of the API, and dissolution characteristics of the dosage form, the BCS approach provides an opportunity to waive *in vivo* pharmacokinetic bioequivalence testing for certain categories of immediate-release drug products.

WHO Definitions

- The highest dose is soluble in 250 ml or less of aqueous media over the pH range of 1.2–6.8 @37° C
- An API is considered highly permeable when the extent of absorption in humans is 85%
- Very rapidly dissolving >85% of the labeled amount in 15 minutes
- Rapidly dissolving >85% of the labeled amount in 30 minutes

WHO Guide: Biowaiver

BCS class 1drugs *are eligible if they dissolve rapidly*

BCS Class 3 drug products, if the multisource and comparator product are very rapidly dissolving

BCS Class 2 weak acids if the API has a dose : solubility ratio of 250 ml or less at pH 6.8 and the multisource product is rapidly dissolving

AND the dissolution profile is similar to that of the comparator product at pH 1.2, 4.5 and 6.8

Similarity Factor F2

$$f_2 = 50 \bullet \log \left\{ \frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} \left[\bar{R}(t) - \bar{T}(t)\right]^2}{n}}} \right\}$$

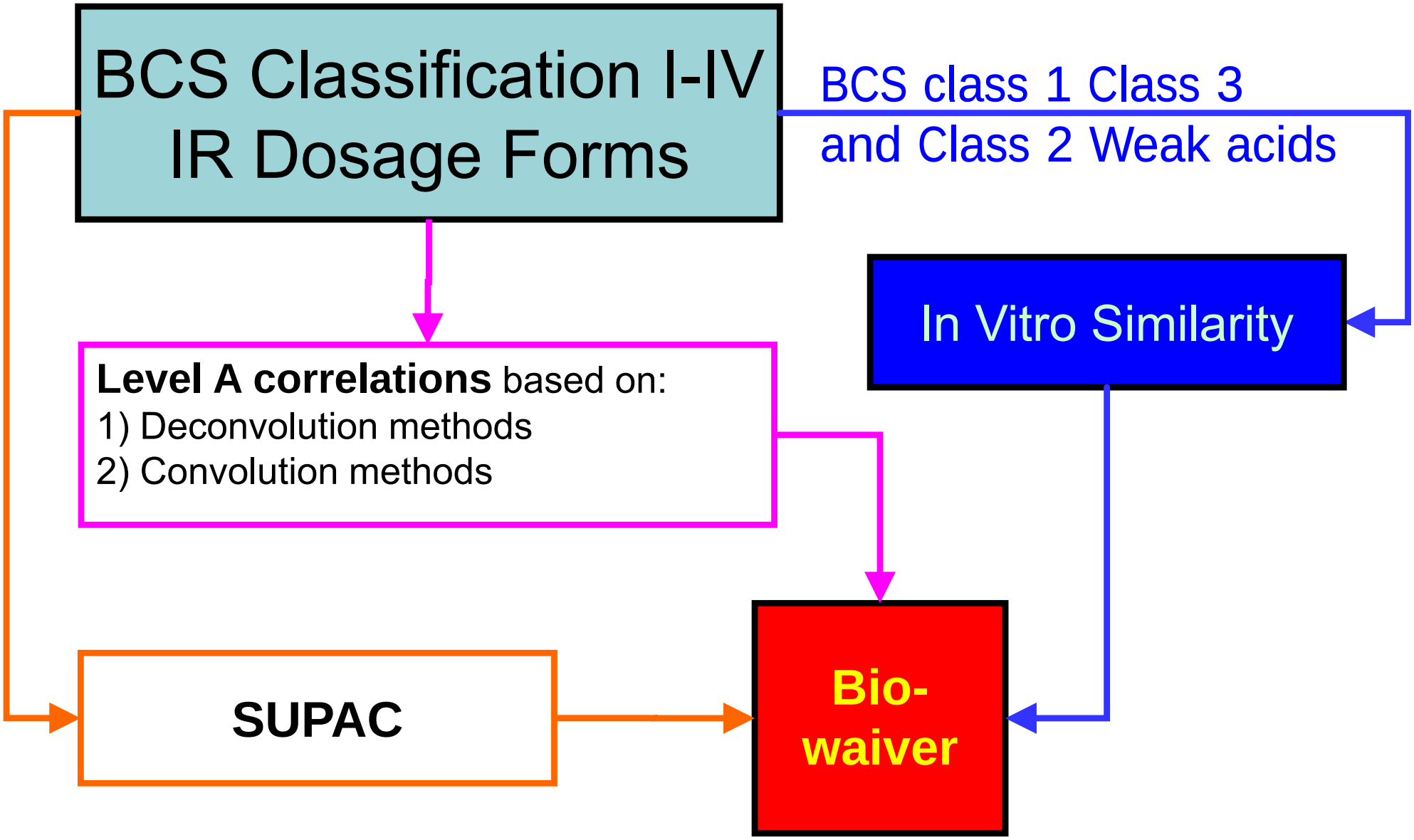
n = number of time points

R(t) = mean % API dissolved of reference product at time point

T(t) = mean % API dissolved of test product at time point

- Minimum of 3 time points (zero excluded)
- 12 units for each product (for “official” purposes)
- Only one measurement should be considered after both products have reached 85 % dissolution
- RSD at higher time points ≤ 10% at the first time point up to 20%

How to get a Biowaiver



WHO Biowaiver for Generics

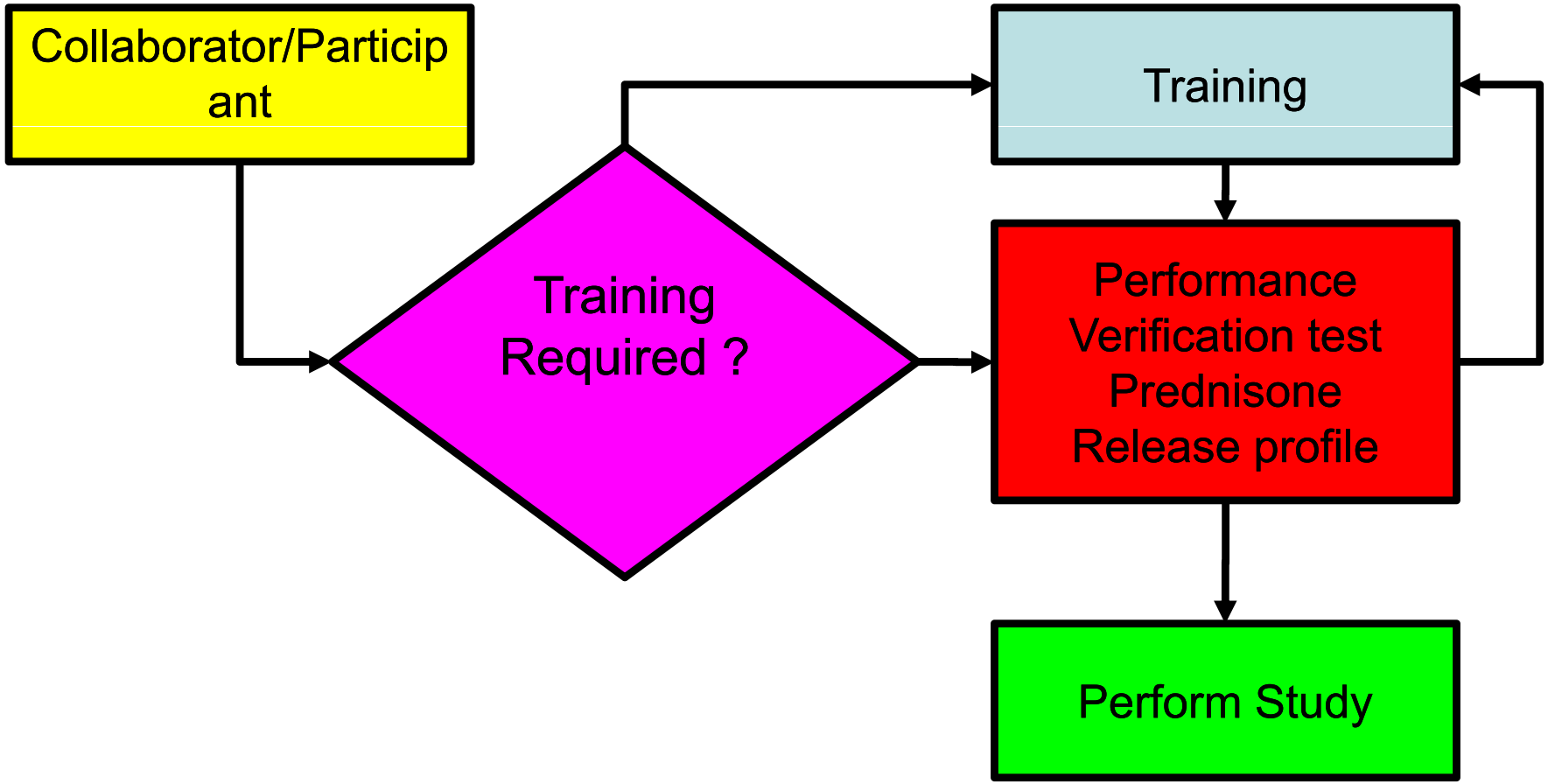
- The innovator product or an assigned listed reference product is the “gold standard”
- Pharmaceutical Equivalence together with *In Vitro* Similarity (IVS) are considered Bioequivalent/Therapeutic Equivalent
- Generics which have similar dissolution profiles (f2) might get market authorization without *in vivo* bioequivalence testing

Study Introduction

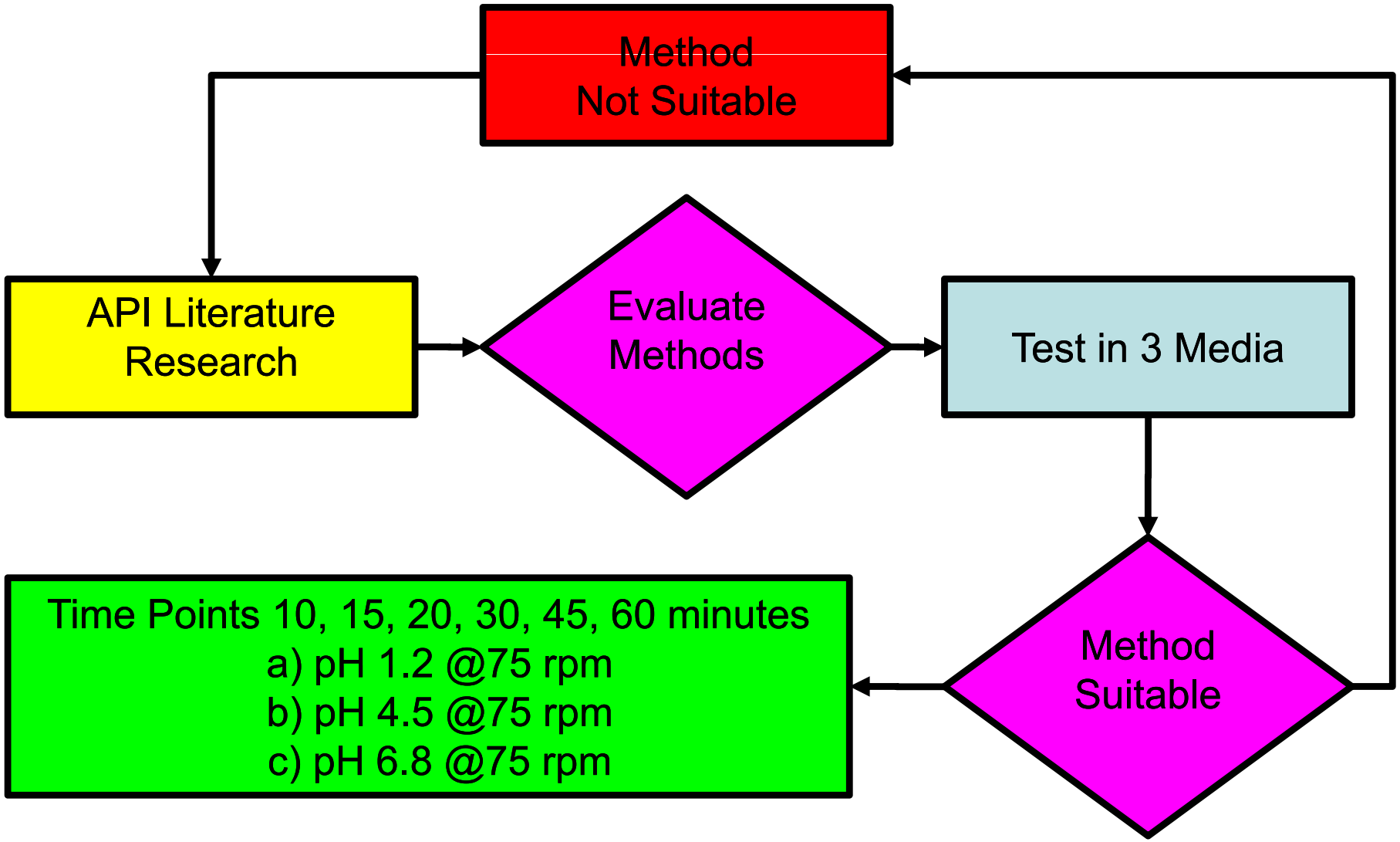
- The study was designed to investigate in vitro dissolution differences between BCS class 1 drugs on the South American Market.
- The products were compared to US products
- A study protocol was developed for three drugs
  - Zidovudine
  - Amoxicillin
  - Metronidazole

The US-RLD was identified and if appropriate an alternative comparator product was chosen

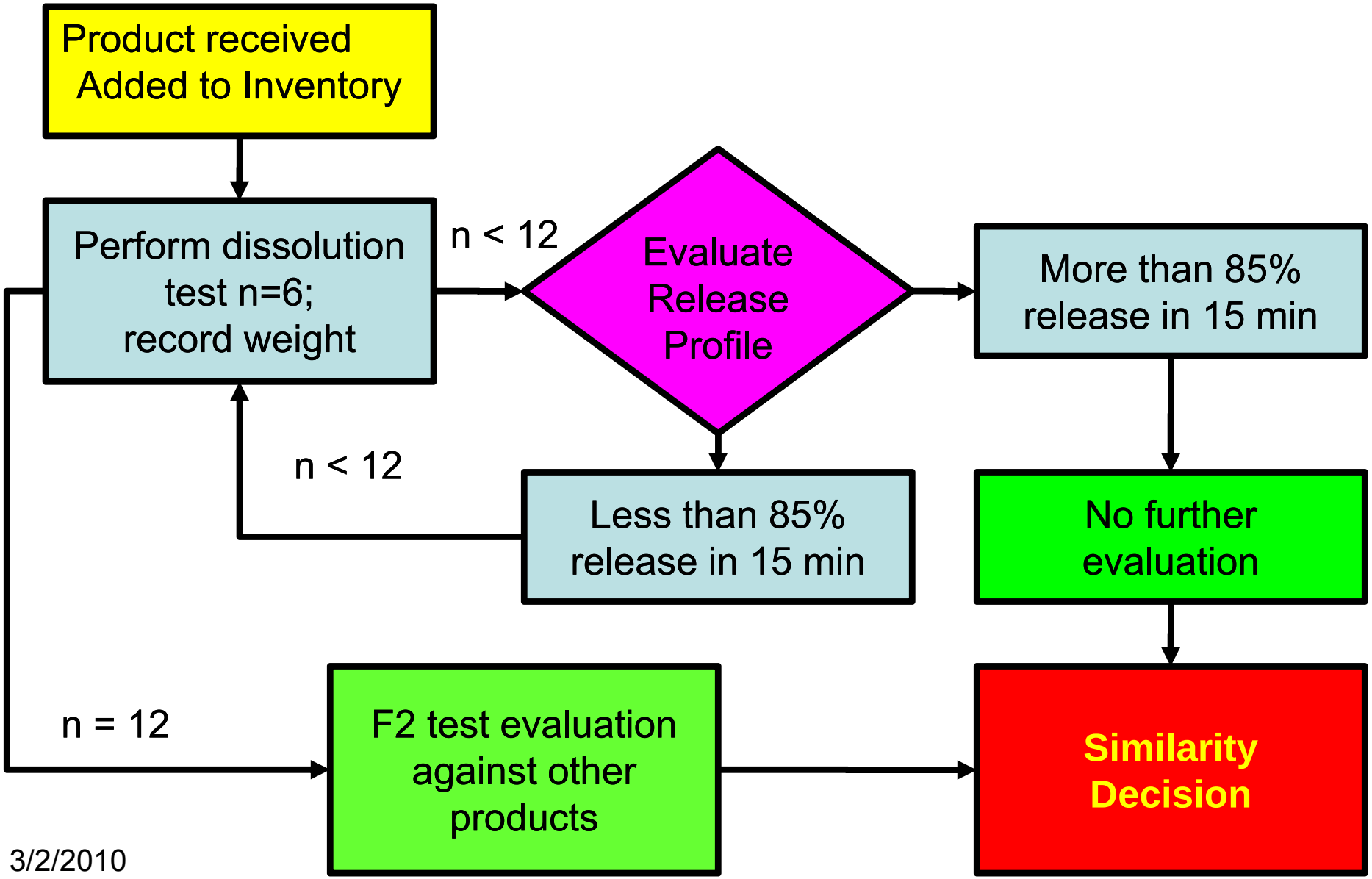
Study Design and Considerations



Study Design and Considerations



Study Design and Considerations



pH 1.2 Buffer v.s. SGF

Gastric Fluid, Simulated, TS—Dissolve 2.0 g of sodium chloride and *3.2 g of purified pepsin, that is derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protei*n, in 7.0 mL of hydrochloric acid and sufficient water to make 1000 mL. This test solution has a pH of about 1.2.

Buffer pH 1.2
0.75 g KCl
7.2 mL HCl

SGF
2.0 g NaCl
7.0 mL HCl

HCl, **36.46 ad 1000 mL (0.2 N / 5 = 7.2 mL)**

14.91 g of potassium chloride (KCl) in water, and dilute with water to 1000 mL.

Composition of Standard Buffer Solutions *Hydrochloric Acid Buffer* Place 50 mL of the potassium chloride solution in a 200-mL volumetric flask, add the specified volume of the hydrochloric acid solution, then add water to volume.

pH1.2 HCl 85 mL

SIF vs. Buffer pH 6.8

Intestinal Fluid, Simulated, TS —Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, and add 77 mL of 0.2 N sodium hydroxide and 500 mL of water. Adjust the resulting solution with either 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 6.8 ± 0.1. Dilute with water to 1000 mL.

NaOH = 0.62 g	NaOH = 0.90 g
KH_2PO_4 = 6.80 g	KH_2PO_4 = 6.81 g

Buffer pH 6.8: Place 50 mL of the monobasic potassium phosphate solution in a 200-mL volumetric flask, add the specified volume of the sodium hydroxide solution, then add water to volume.

pH						
5.8	6.0	6.2	6.4	6.6	6.8	7.0
0.2 M NaOH, mL						
3.6	5.6	8.1	11.6	16.4	22.4	29.1

Media Preparation

The media are prepared according to USP 1.

A microwave is used to heat the media 2.

The warm media were de-aired by filtering them into a bottle immerged into an ultrasonic bath 3.

Dissolution Apparatus Set-up

The media are weighed into the dissolution vessels and the dissolution apparatus is set up for the test ❶.

An auto-sampler is used to collect 1 mL samples ❷.

Analyze and Clean-up

After the test the vials are transferred

to an HPLC system ❶.

The dissolution apparatus was cleaned with a semi automated

washing in place system ❷.

Zidovudine's footprint

USP Requirements

Zidovudine Capsules

Mode: LC
Detector: UV 265 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability

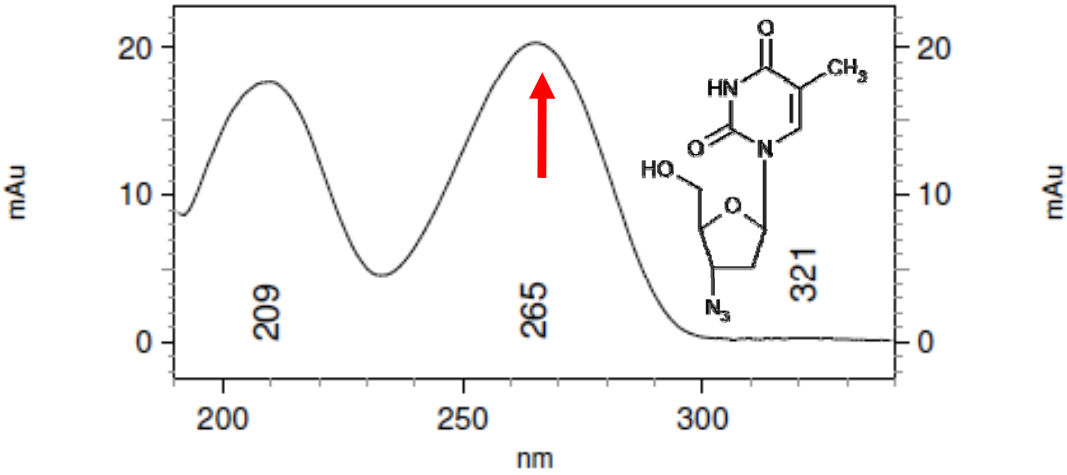
Dissolution

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Tolerances: NLT 75% (Q).
Uniformity of Dosage Units 905

Analytical Method

Method

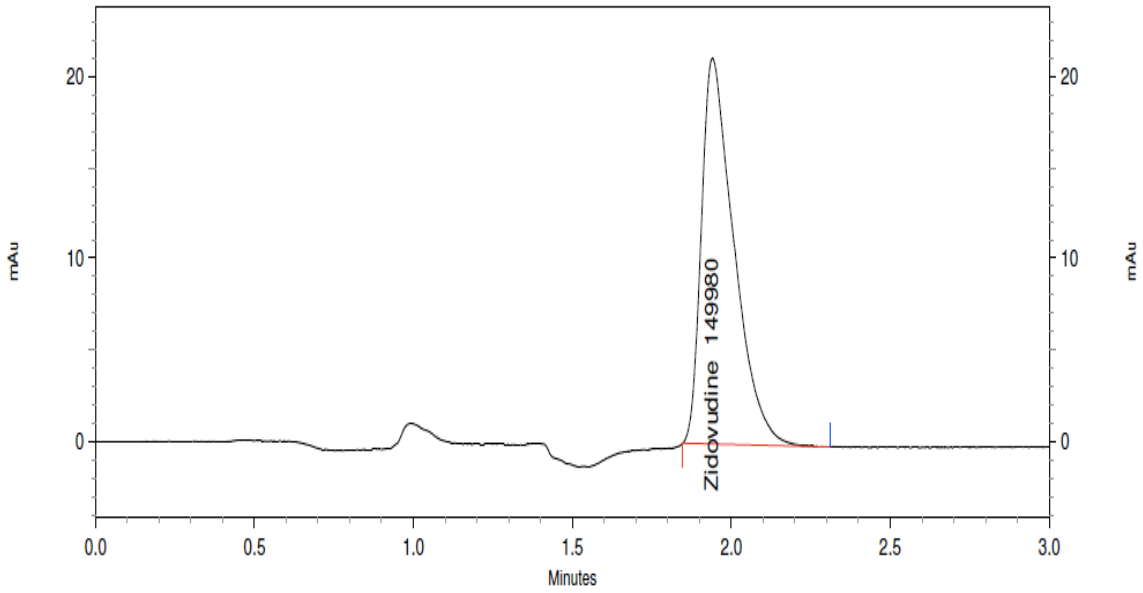
Mode: LC
Detector: UV 265 nm
Column: RP-8
Water : ACN mixture (72:28)
Flow rate: 1 mL/min
Injection size: 10 µL



Low concentration

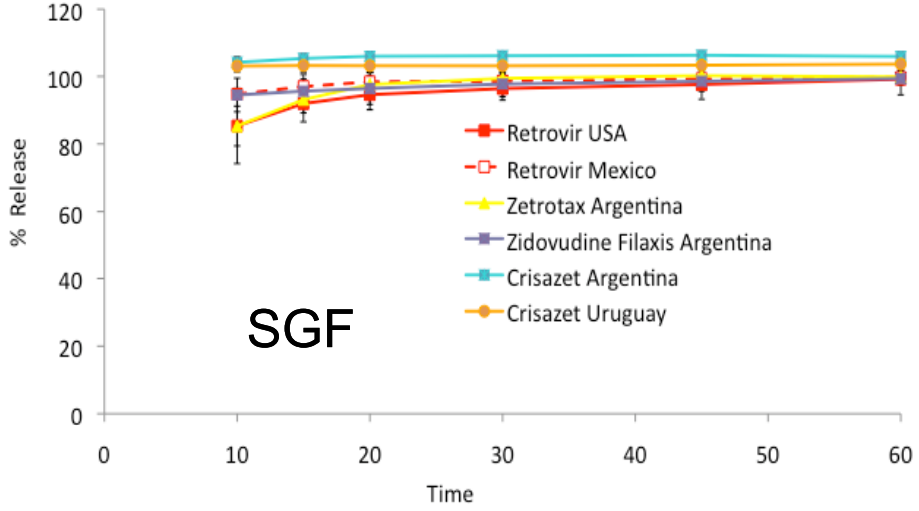
Dissolution

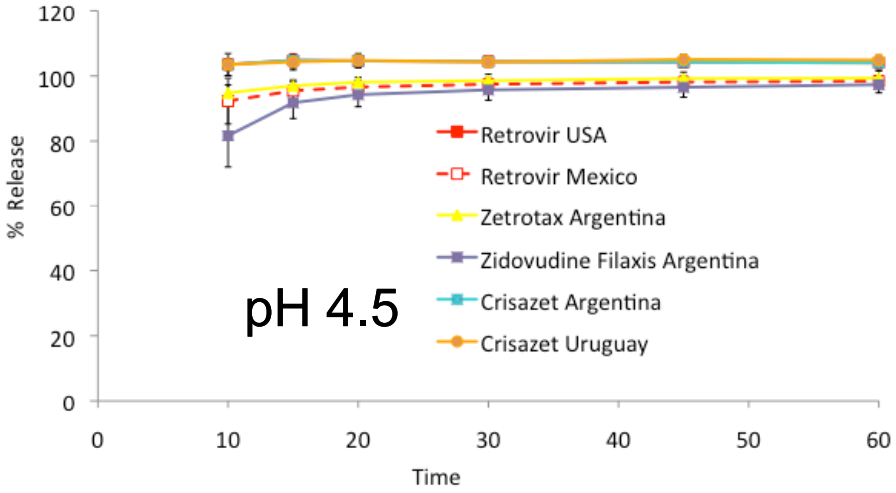
Apparatus 2 : 75 rpm
Time: 10, 15, 20, 30, 45, 60 min
Media: SGF, pH 4.5, SIF

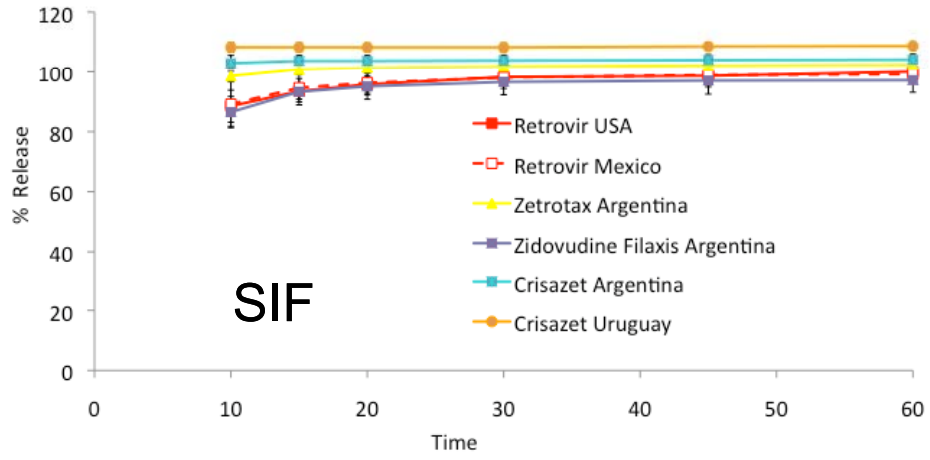


Zidovudine

In Vitro Similarity (IVS)

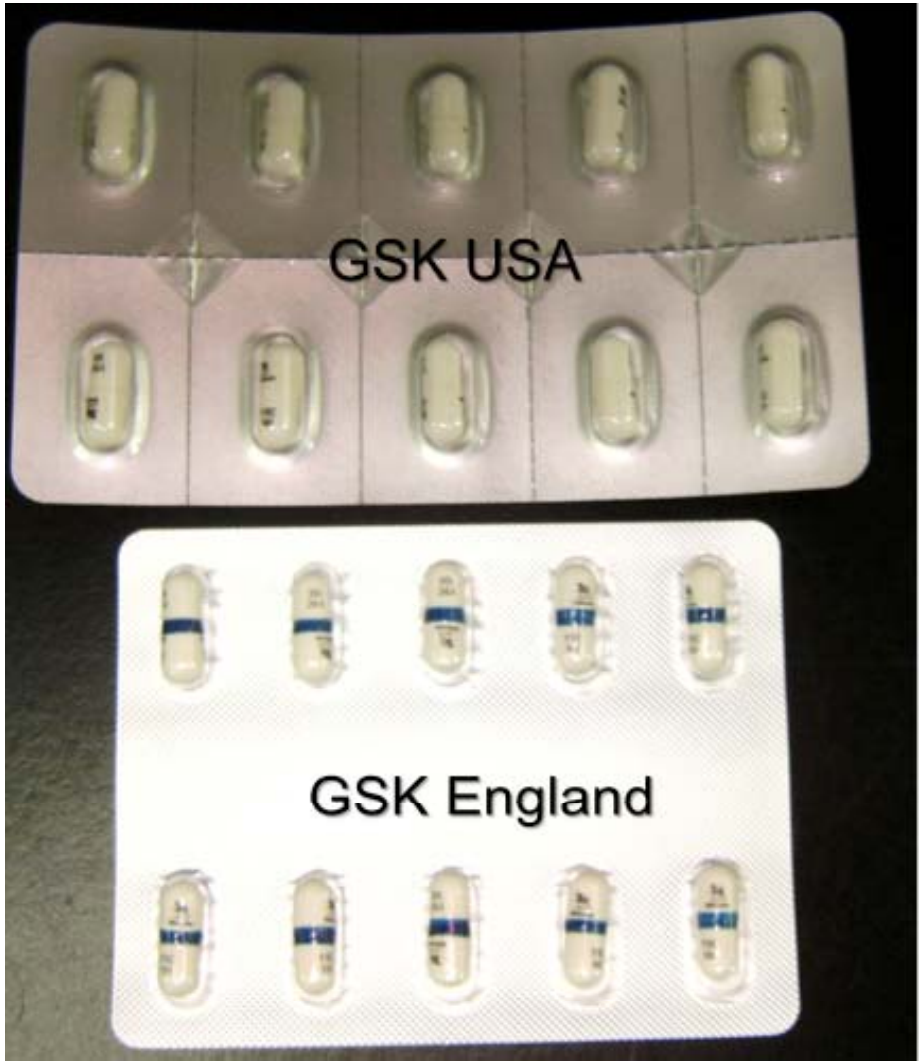


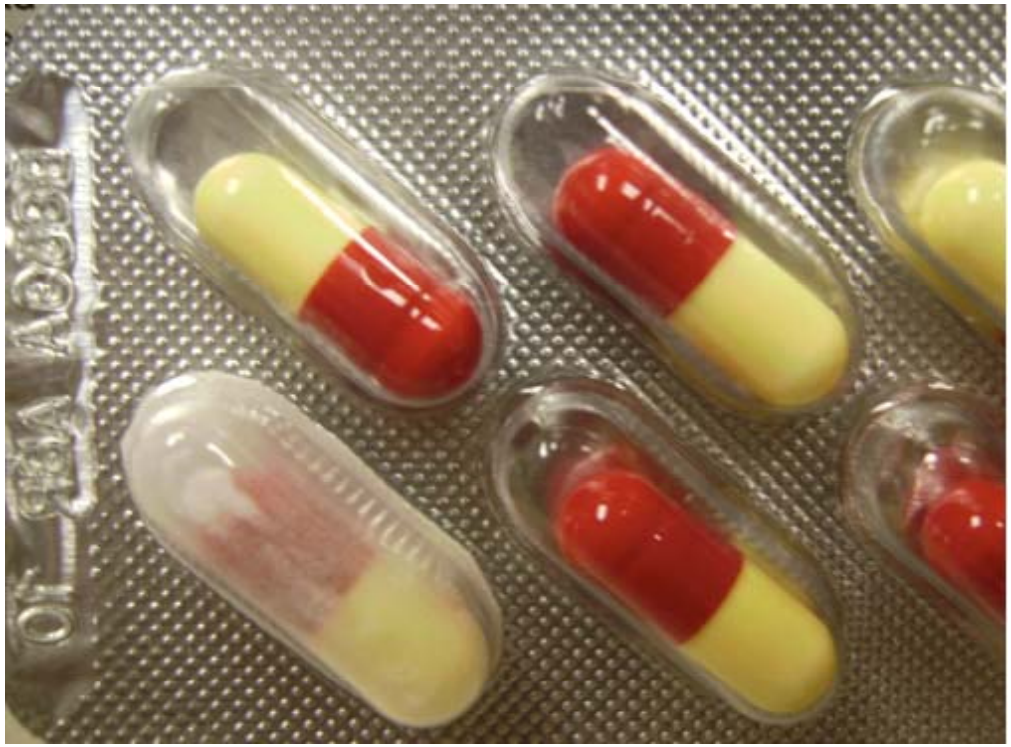




Country	Company	Product	Batch	Exp.	Excipient
USA	GSK USA	Retrovir	7ZP 1642	10/10	Corn Starch, Mg-Stearate, MCC, Sodium Starch Glycolate
Mexico	GSK (England)	Retrovir	X5953	05/10	NA
Argentina	Laboratorios Richmonds	Zetrotax	EMX 4V	04/10	Excipients
	Laboratoris Filaxix	Zidovudina	12119 D1	06/10	Lactose monohydrate, Mg-Stearate, MCC, Cross carmelose Sodium, Silicium Dioxide
	Laboratorio LKM	Crisazet	B853A	04/10	Sodium Starch Glycolate, Lactose Monohydrate, Mg-Stearate
Uruguay	Laboratorio LKM	Crisazet	B853A	04/10	Sodium Starch Glycolate, Lactose Monohydrate, Mg-Stearate

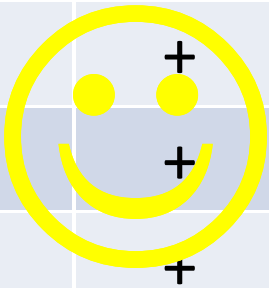
Packaging and Manufacturing differences and deficiency





Summary Zidovudine Dissolution comparison vs. Retrovir (US-RLD)

Country	Name	SGF	pH 4.5	SIF
Mexico	Retorvir	+	+	+
Argentina	Crisazet	+	+	+
	Zetrotax	+	+	+
	Filaxix	+	+	+
Uruguay	Crisazet	+	+	+



The tested products are

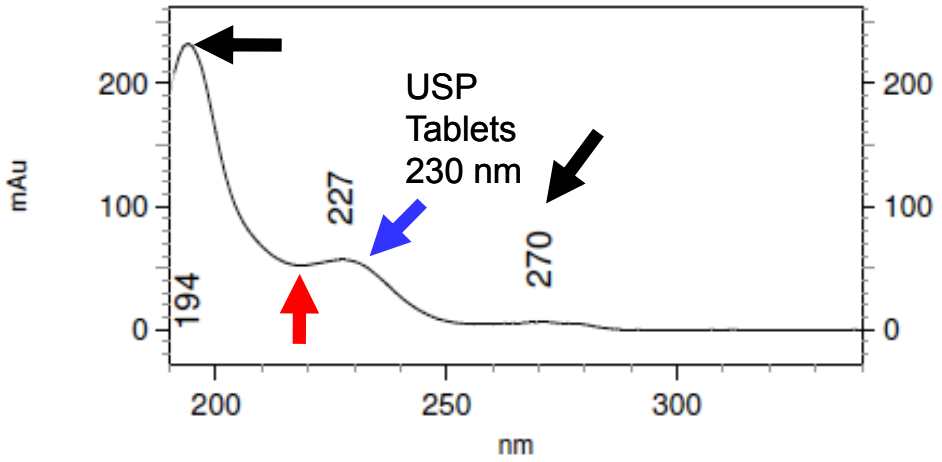
- Pharmaceutically Equivalent
- *In Vitro* Similarity (IVS)
- All investigated products pass the WHO guideline for *in vitro* Therapeutic Equivalence

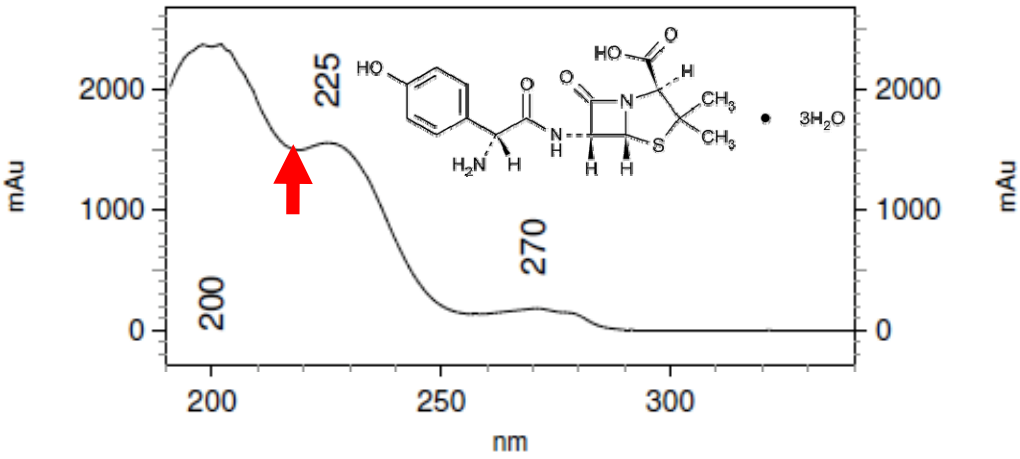
Mapping

Amoxicillin Tablets USP 32

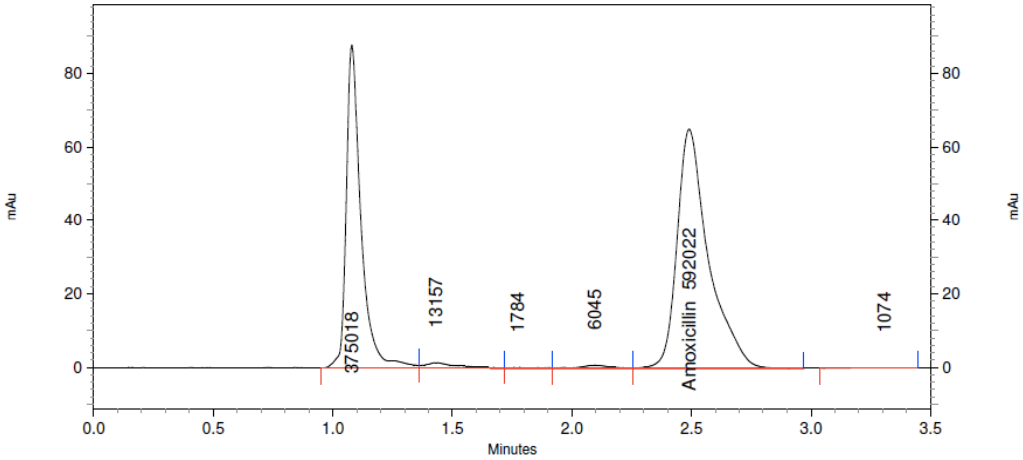
- Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount
- Dissolution: water; 75 rpm, App 2
- Q75% @ 30 min
- No Content Uniformity tests!

Amoxicillin Analytical Development

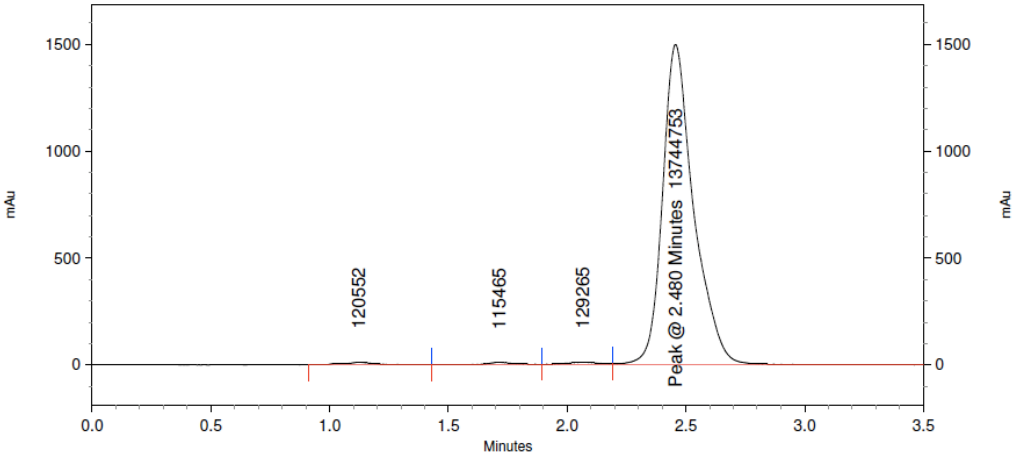




Low concentration



High concentration



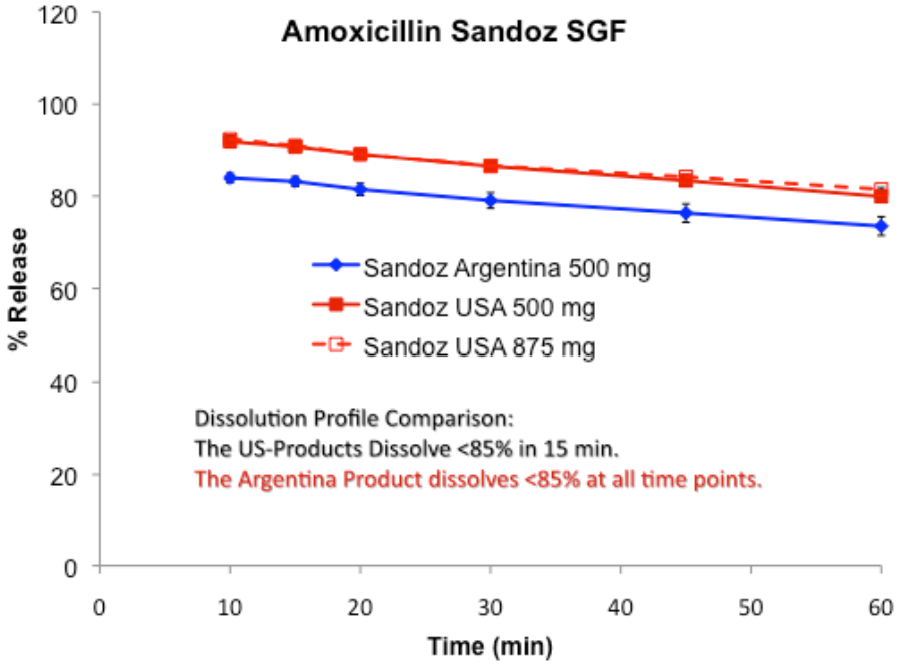
Study Design

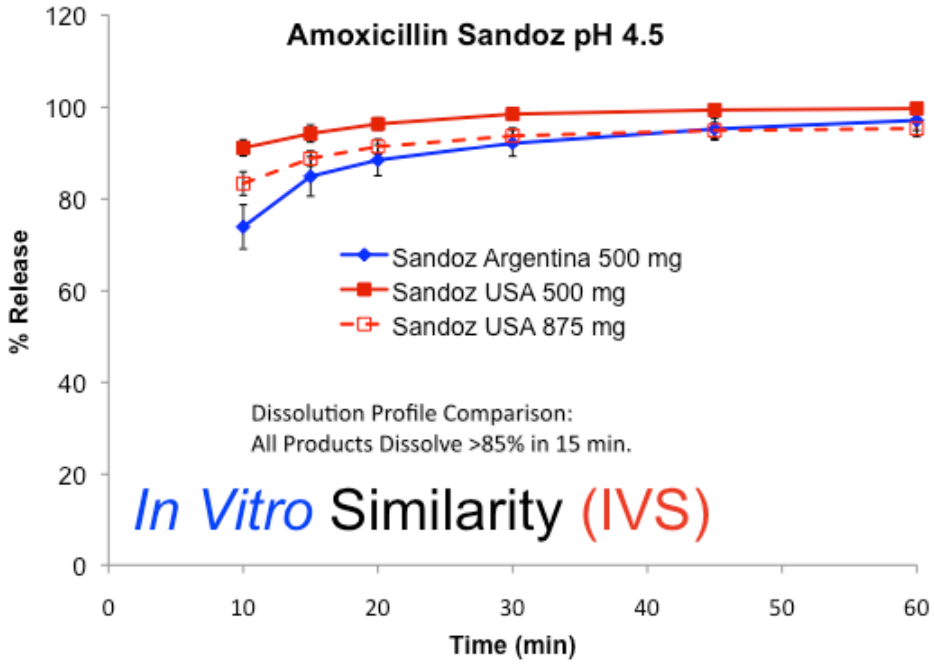
- Analytical assay
  - HPLC 219 nm
  - RP 18 Column
  - 1 mL/min Acetonitrile : Buffer, pH 5.0
  - Retention time about 3 min
- App 2, 75 rpm
  - SGF, buffer pH 4.5, SIF
- Time points
  - 10,15, 20, 30, 45, 60

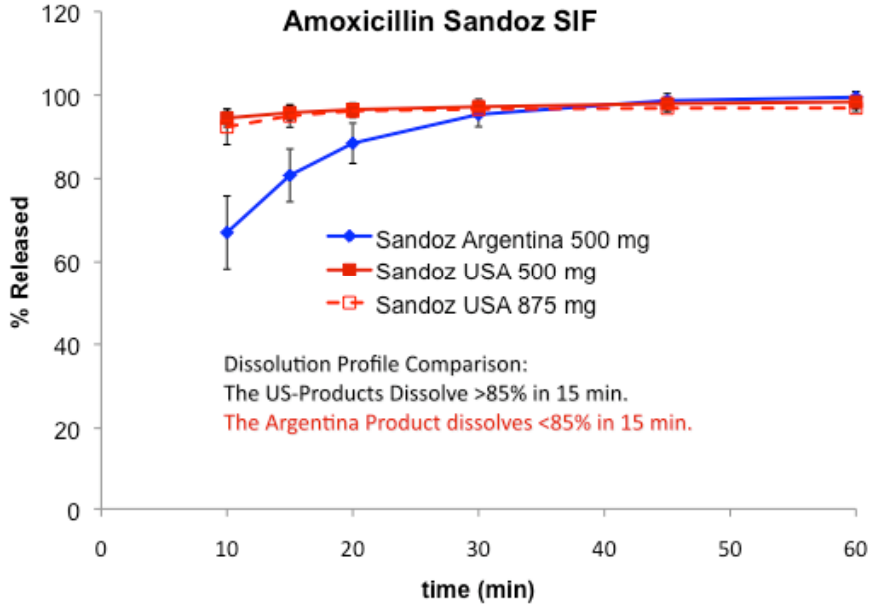
Commentary

- The US products are dose proportional products (875 mg and 500 mg)
- Sandoz produces products sold in the US and Argentina in the same factory in Austria
- The Argentina product has different excipients compared to the US products

Sandoz Products

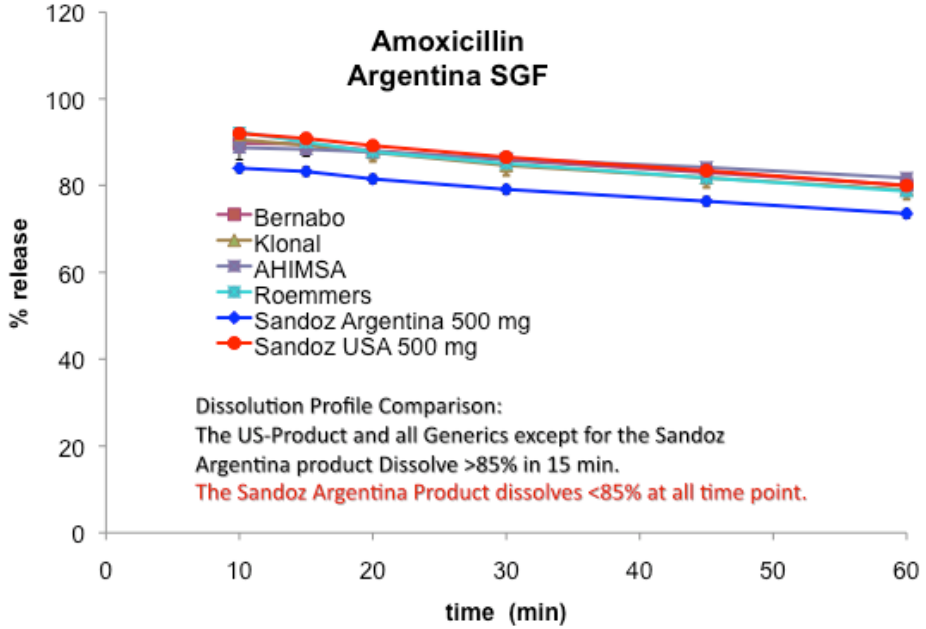


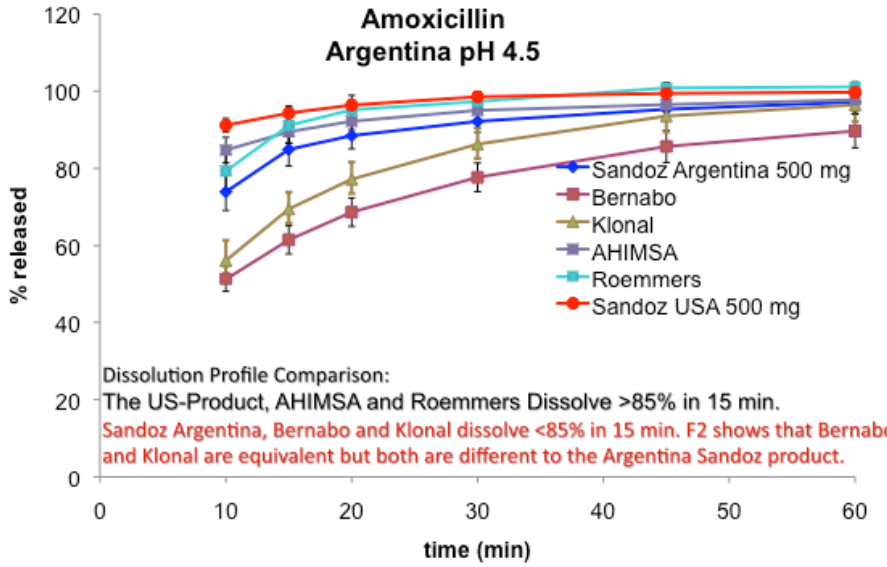


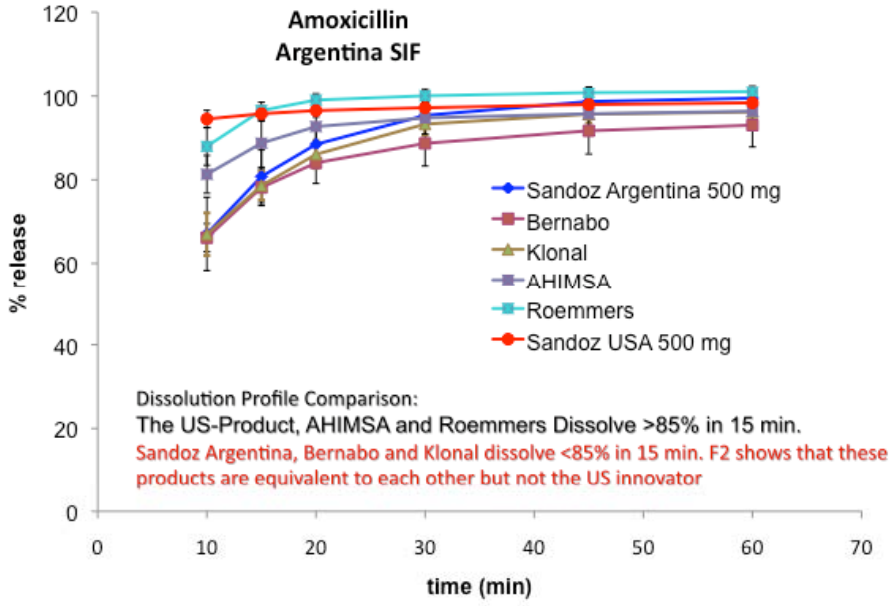


Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin 875/500 mg	147419 151645	03/09 10/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, ~~MG-Stearate~~, ~~MCC,~~ Sodium Stach glycolate, talc, triethy.citrate, ~~titanium dioxide~~
Argentina	Sandoz	Telmox 500 mg	00018	01/11	magnesium stearate; ~~micro~~ crystalline cellulose; ~~titanium dioxide~~; *~~hydroxy~~propylcellulose; povidone; sodium carboxy* methyl *starch*

Argentina





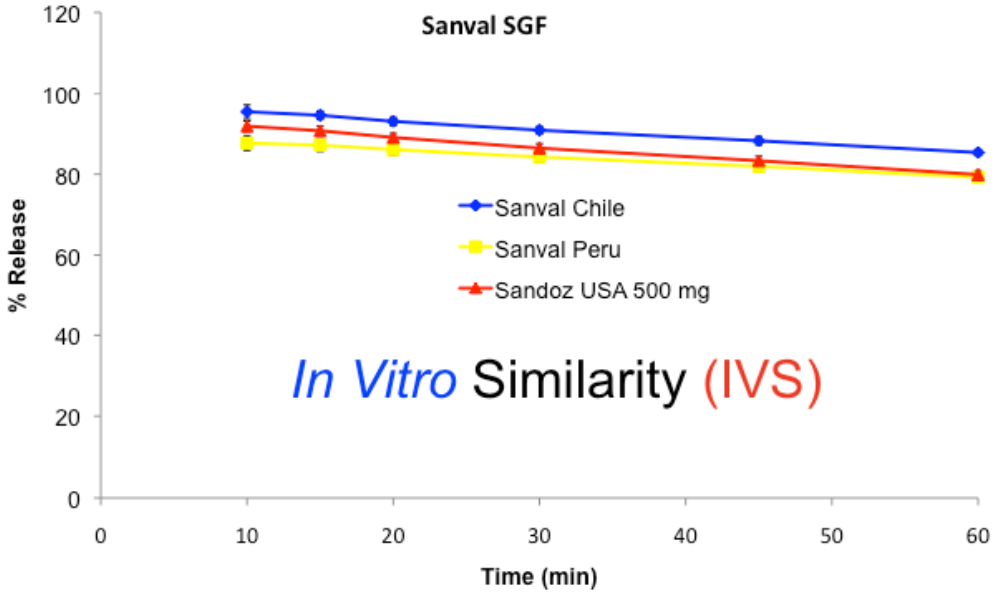


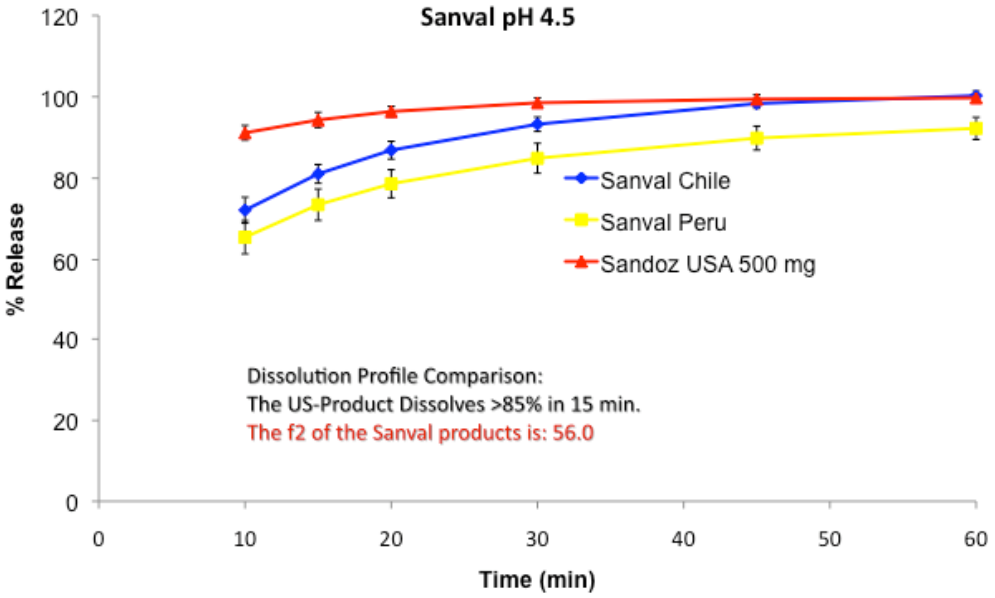
Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin 500 mg	151645	10/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, MG-Stearate, MCC, Sodium Stach glycolate, talc, triethy.citrate, titanium dioxide
Argentina	Roemmers	Amoxidal	00633	11/10	starch; crospovidone; sodium lauryl sulfate; magnesium stearate; micro crystalline cellulose; hypromellose; titanium dioxide; polyethylene glycol ; triacetine; corante
	Klonal	Amox - G	A5802	01/10	Authorized excipients
	Bernabo	Amixen 500 mg	117183	11/09	hypromellose; polyethylene glycol; crospovidone; magnesium stearate; micro crystalline cellulose; lactose; titanium dioxide; triacetine; amaranthus
	AHIMSA	Amoxigrand	P213G911	10/10	Authorized Excipients
	Sandoz	Telmox 500 mg	00018	01/11	magnesium stearate; micro crystalline cellulose; titanium dioxide; *hydroxypropylcellulose; povidone; sodium carboxy* methyl *starch*

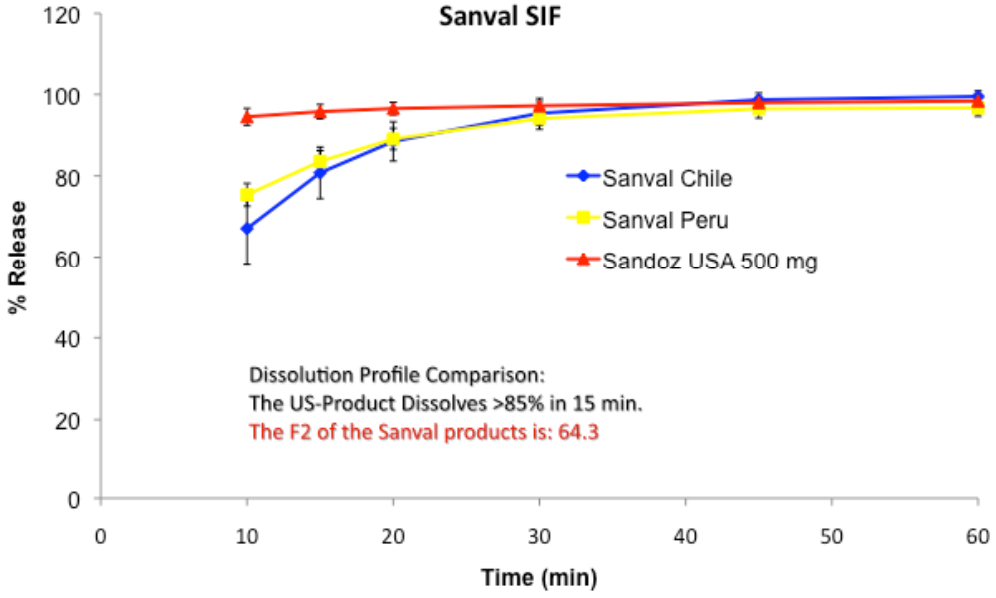
Commentary

- Sanval is a South American Company which has market authorization for the same product in different countries.
  - Two different batches from different countries were compared to the RDL and to each other…

Same Product from different Countries





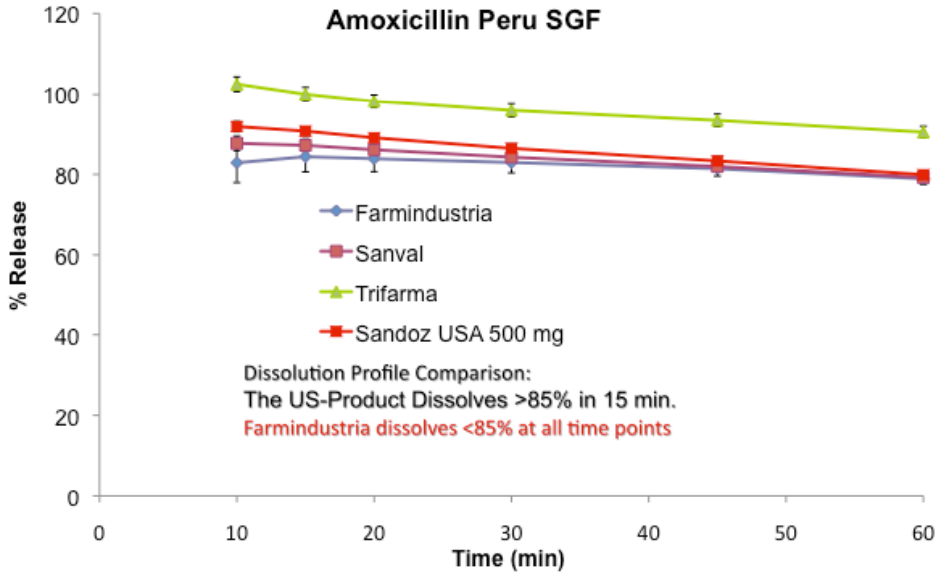


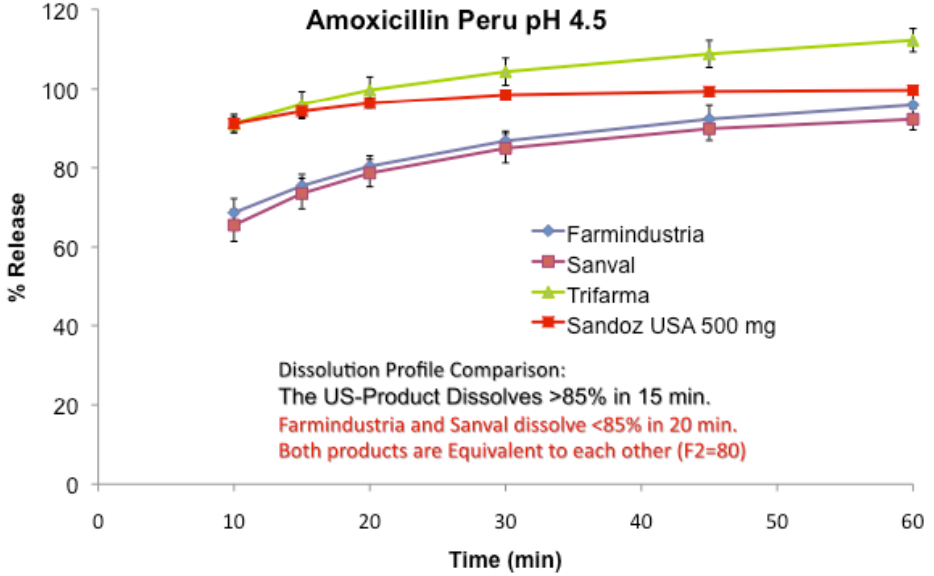
Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin	151645	10/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, MG-Stearate, MCC, Sodium Stach glycolate, talc, triethy.citrate, titanium dioxide
Peru	Sanval	Amoval 500 mg	122387	07/12	croscarmellose sodium; micro crystalline cellulose; magnesium stearate; titanium dioxide; polyethylene glycol; hypromellose; *Eicosadioate*
Chile	Sanval	Amoval 500 mg	033608	11/2012	croscarmellose sodium; micro crystalline cellulose; magnesium stearate; titanium dioxide; polyethylene glycol; hypromellose; *Eicosadioate*

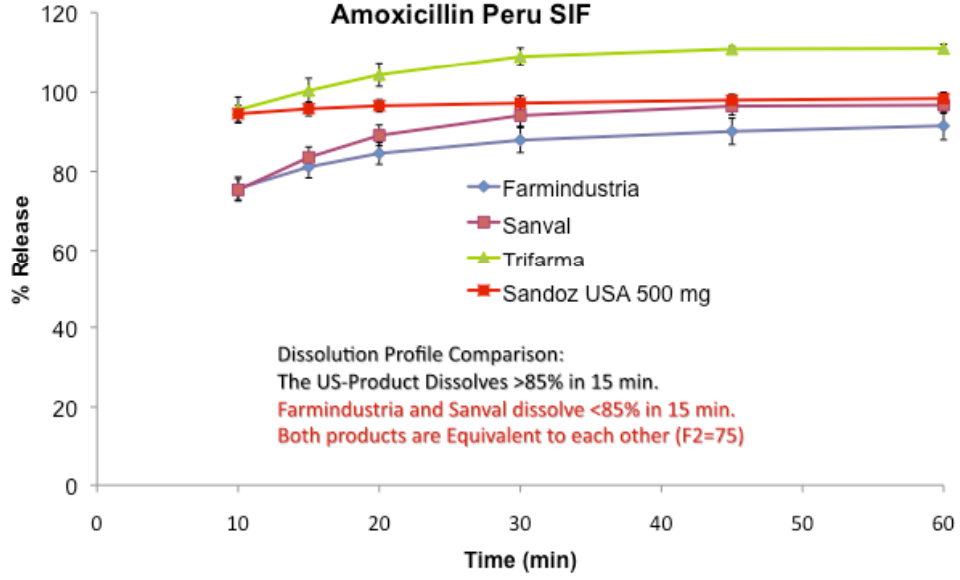
In Vitro Similarity (IVS)

- The two Sanval products tested have similar drug release profiles to each other but fail the comparison with the US product

Amoxicillin Peru

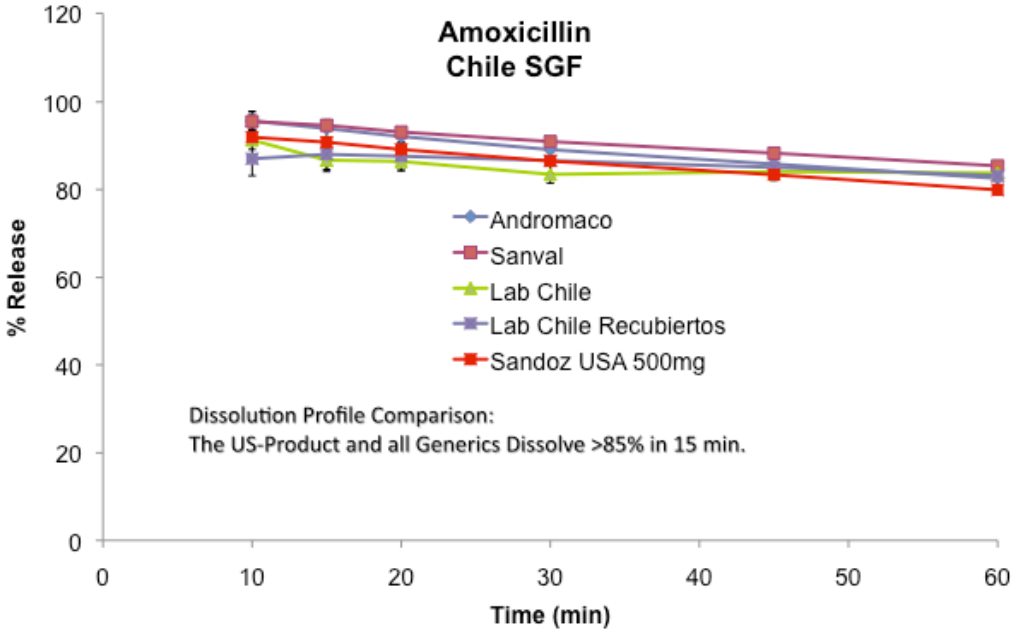


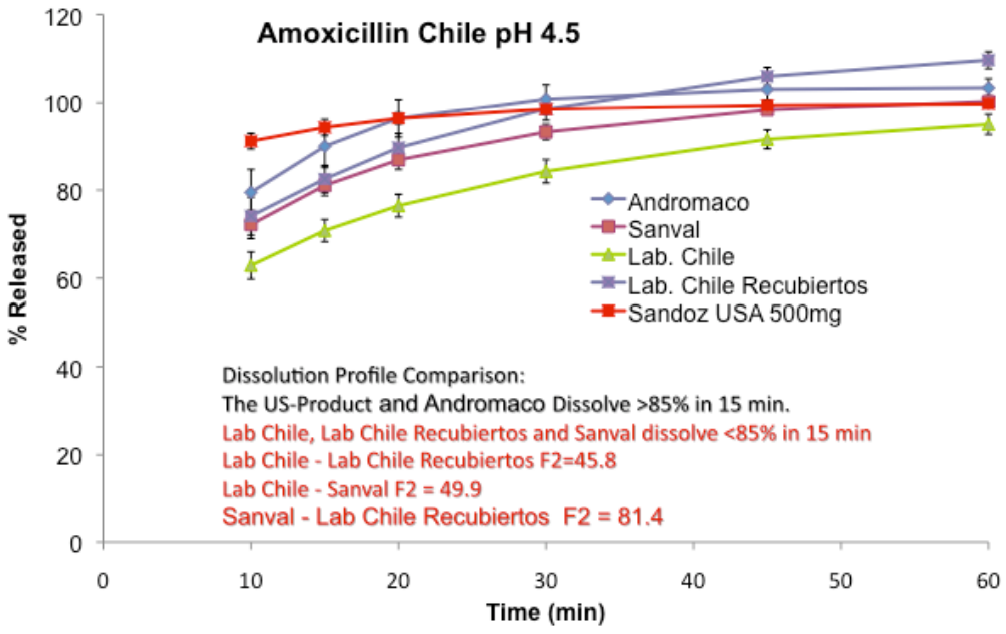


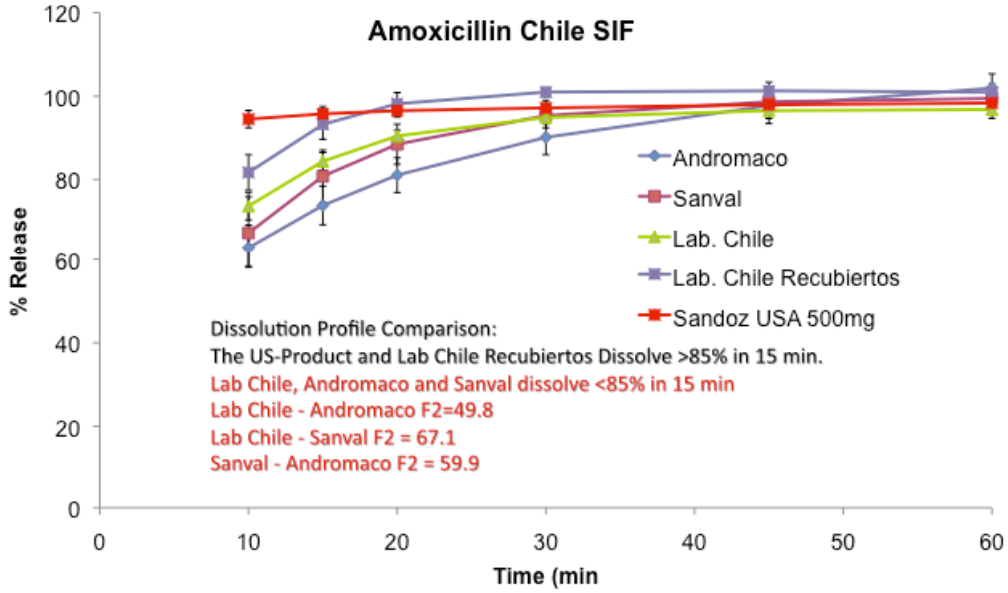


Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin	151645	10/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, MG-Stearate, MCC, Sodium Stach glycolate, talc, triethy.citrate, titanium dioxide
Peru	Sanval	Amoval	122387	07/12	croscarmellose sodium; micro crystalline cellulose; magnesium stearate; titanium dioxide; polyethylene glycol; hypromellose; *Eicosadioate*
	Grünenthal (Trifarma)	Grunamox	009016	09/09	Excipients
	Farmindustria	Amoxicilina	00921787	09/10	Excipients

Amoxicillin Chile







Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin 500 mg	151645	10/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, MG-Stearate, MCC, Sodium Stach glycolate, talc, triethy.citrate, titanium dioxide
Chile	Laboratórios Chile	Amobiotic	08016317	01/11	povidone; sodium starch glycolate; micro crystalline cellulose; magnesium stearate; polymeric coating; talc; titanium dioxide; simeticone; macrogol; hypromellose
		Amoxicilina	07072912	07/10	Excipients
	Andromaco	Amoxicilina	1700408	12/09	Excipients
	Sanval	Amoval 500 mg	033608	11/2012	croscarmellose sodium; micro crystalline cellulose; magnesium stearate; titanium dioxide; polyethylene glycol: hypromellose: *Eicosadioate*

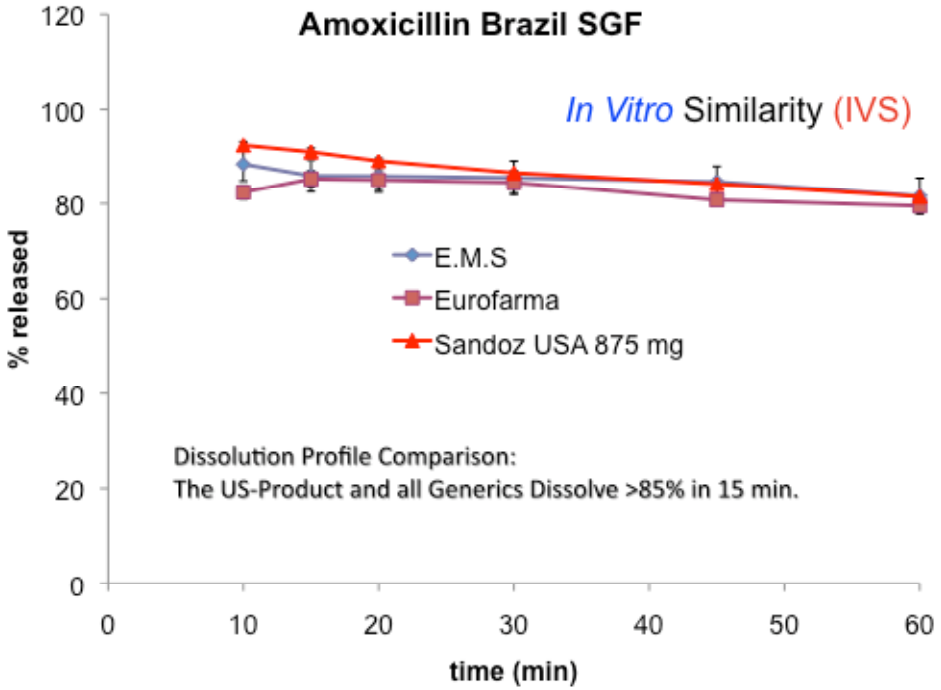
Summary Amoxicillin Dissolution comparison vs. Sandoz (US 500 mg)

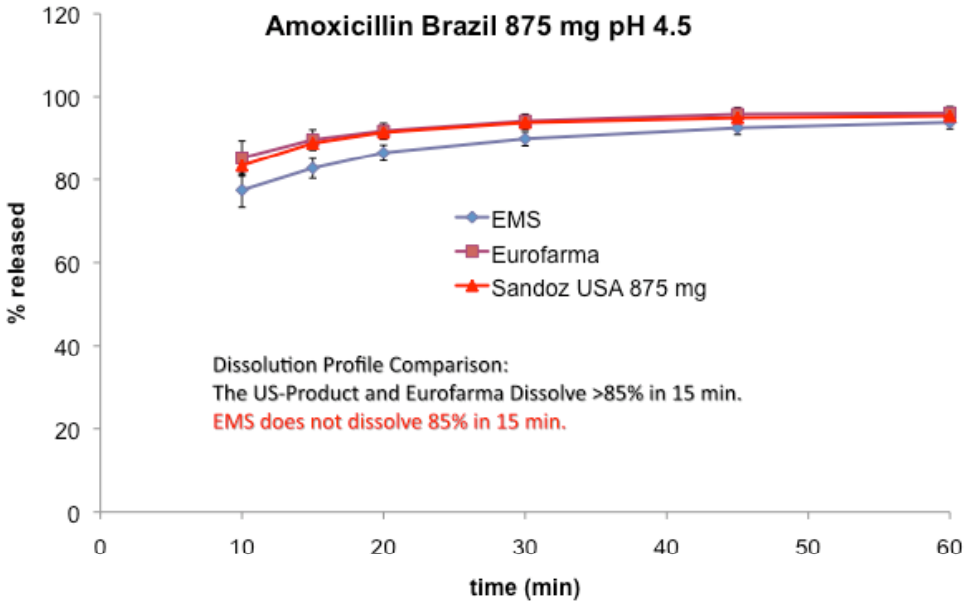
Country	Manufacturer	SGF	pH 4.5	SIF
Argentina	Sandoz	-	-	-
	Bermabo	+	-	-
	Klonal	+	-	-
	AHIMSA	+	+	+
	Roemmers	+	+	+
Peru	Sanval	+	-	-
	Trifama	+	+	+
	Farmindustria	-	-	-
Chile	Sanval	+	-	-
	Andromaco	+	+	-
	Lab Chile	+	-	-
	Lab Chile Recubiertos	+	-	+

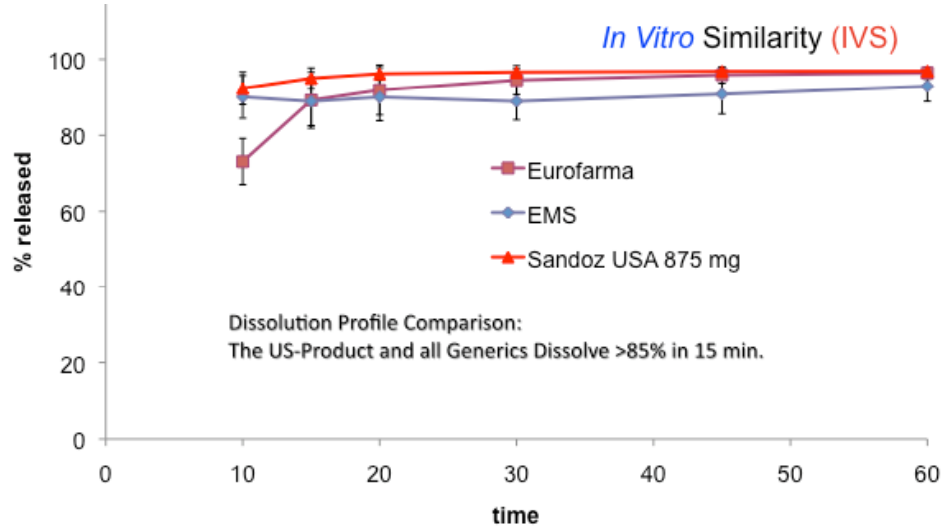
Commentary

- 500 mg is a common dose use in Europe and several countries in South America
- 875 mg is the strength of the US-RLD.
- 875 mg is also used in Brazil

Brazil







Country	Company	Product	Batch	Exp.	Excipients
USA	Sandoz	Amoxicilin 875 mg	147419	03/09	Silicon dioxide, crosspovidone, ethylcellulose aqueous dispersion, hypromellose, MG-Stearate, MCC, Sodium Stach glycolate, talc, triethy.citrate, titanium dioxide
Brazil	EuroFarma	Amoxicillina 875 mg	132000	10/09	Crosspovidone, magnesium stearate, micro crystalline cellulose, titanium dioxide rutilo,, colloidal silicon dioxide, hydroxypropylcellulose-g-poly(ethyleneglyclol); eritrosine
	EMS	Amoxicillina 875 mg	127735	01/2010	Starch, povidone; croscarmellose sodium; sodium starch glycolate; colloidal silicon dioxide; , magnesium stearate; hypromellose; macrogol; titanium dioxide; eritrosine

Summary Amoxicillin Dissolution Comparison vs. Sandoz (US 875 mg)

Country	Manufacturer	SGF	pH 4.5	SIF
Brazil	Europharma	+	+	+
	EMS	+	-	+

Summary Amoxicillin

- 14 generics and two US products (500 and 875 mg amoxicillin) were tested
- 10 generics failed *In Vitro* Similarity (IVS) criteria according to WHO guidelines
  - Three of the products who failed failed only by a margin of about 3%

Zooming in on

USP Metronidazole

API- Procedure

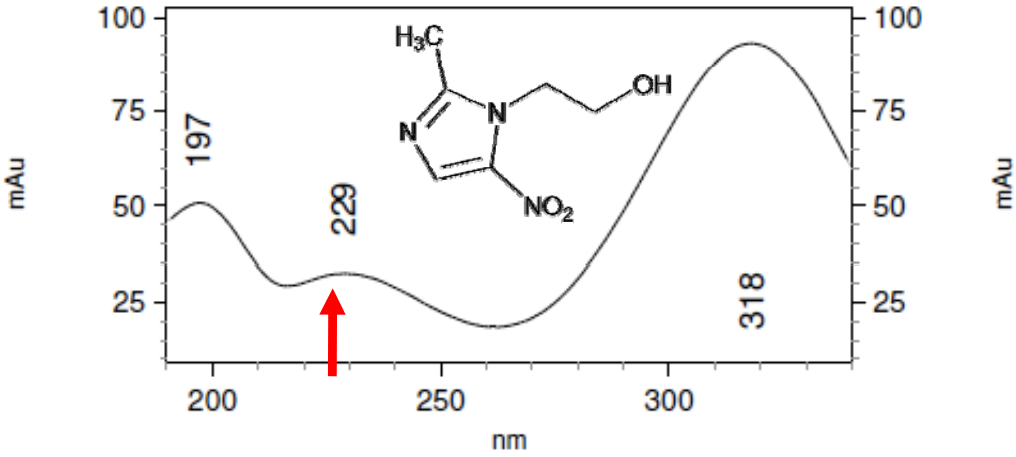
Mobile phase: methanol and water (1:4)
Mode: LC
Detector: UV 319 nm
Column: 4.6-mm × 15-cm column; 5-µm packing L7
Temperature: 30
Flow rate: 1 mL/min

TABLETS

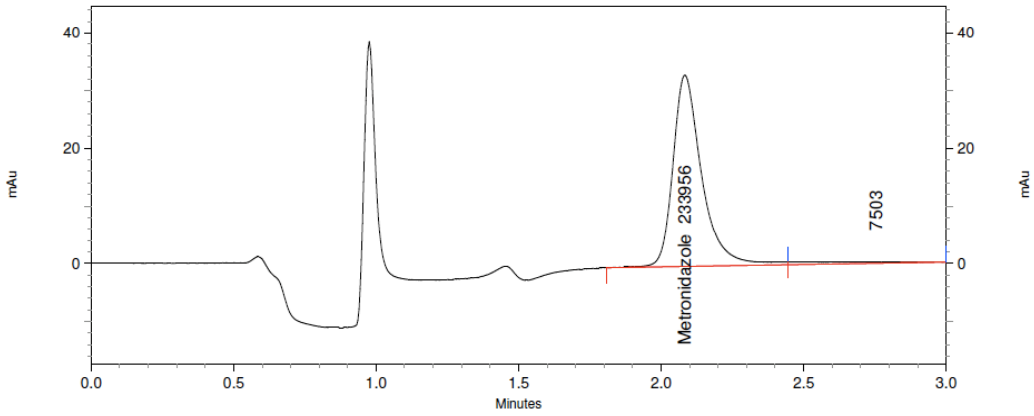
Metronidazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount
Dissolution 711
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Mode: UV Maximum at about 278 nm (Dissolution, Uniformity); Assay at 254 nm
Tolerances: NLT 85% (Q) of the labeled amount.

Analytical Method

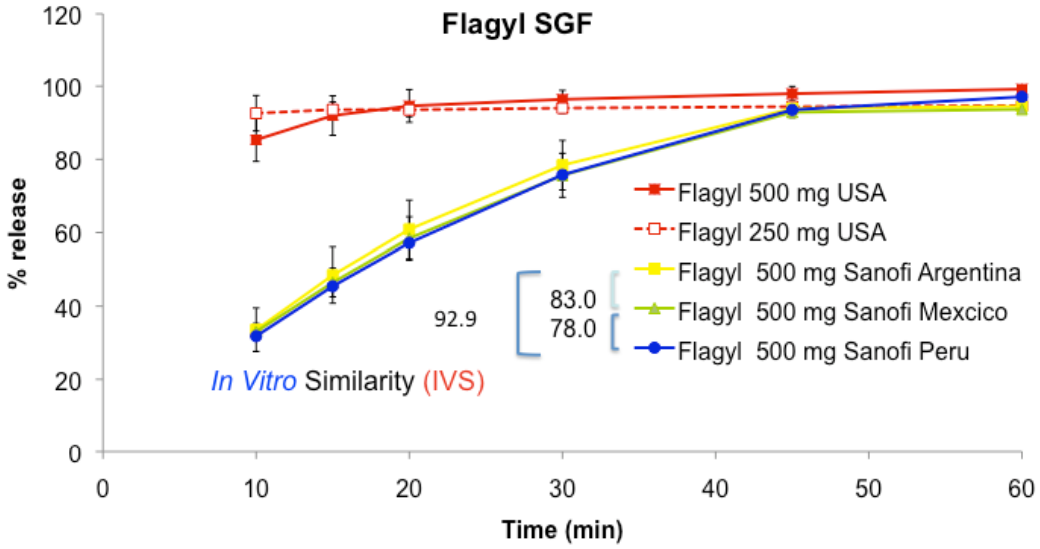
Method
Mode: LC
Detector: UV 228 nm
Column: RP-8
Water : ACN mixture (66:34)
Flow rate: 1 mL/min
Injection size: 10 µL

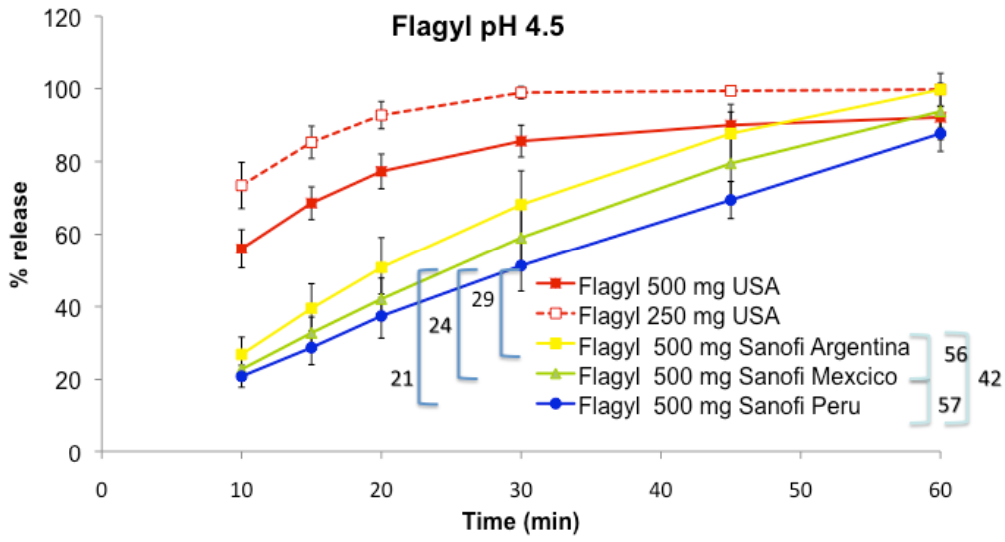


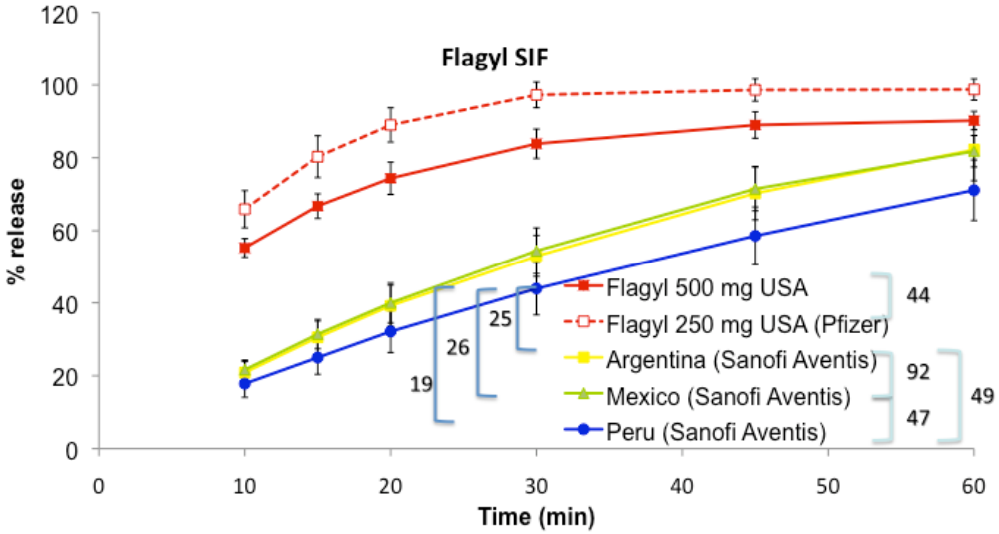
Dissolution
Apparatus 2: 75 rpm
Time: 10, 15, 20, 30, 45, 60 min
Media: SGF, pH 4.5, SIF



Flagyl





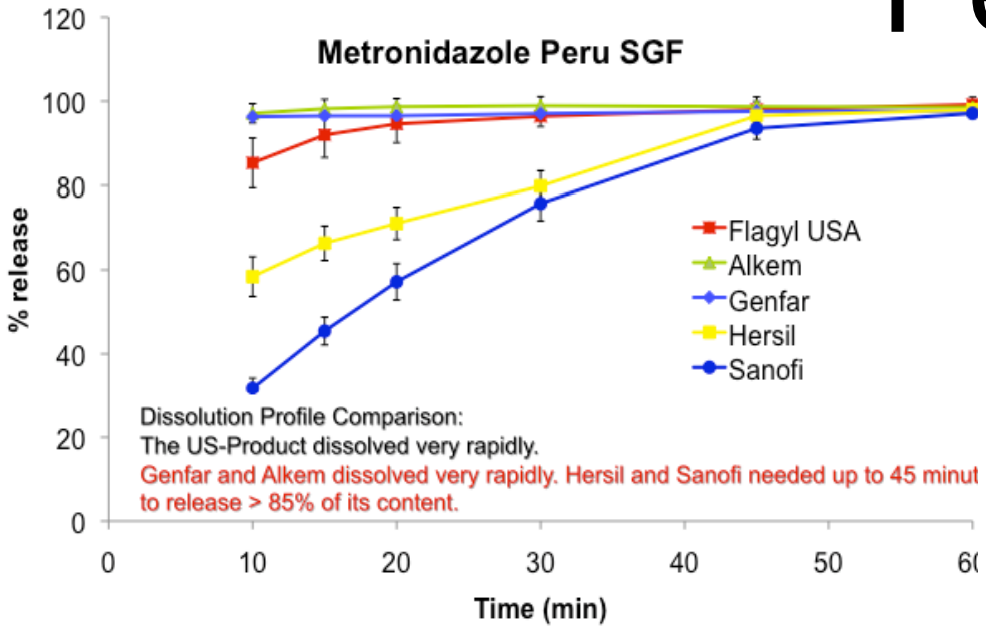


Country	Company	Product	Batch	Exp.	Excipient
USA	Searle Pharmacia Pfizer Searle	500 mg 250 mg	C061228 C071099	03/09 09/10	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
Mexico	Sanofi Aventis	500 mg	B8B575	03/11	Excipients
Argentina	Aventis	500 mg	U6121	10/10	Water, Ethanol, Maize Starch, Calcium Phosphate Dihydrate, Mg-stearate, HMPC, Whithe Wax, titanium Dioxide, PEG 20,000 Polyvidone, Sorbitol Anhydrate
Peru	Sanofi Aventis	500 mg	C8R392	01/11	Excipients

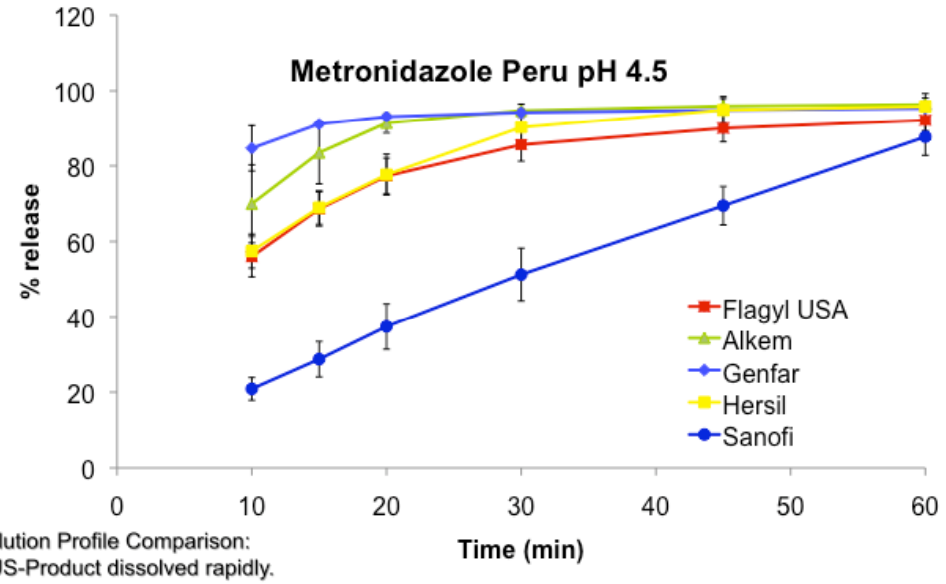
Flagyl

- The US- RLD and the marketed product in South America are made by different companies
- The product manufactured by Sanofi-Aventis is not *In Vitro* Similar (IVS) the RDL.
- Flagyl Peru is failing *In Vitro* Similarity (IVS) with the products received from Argentina and Mexico.

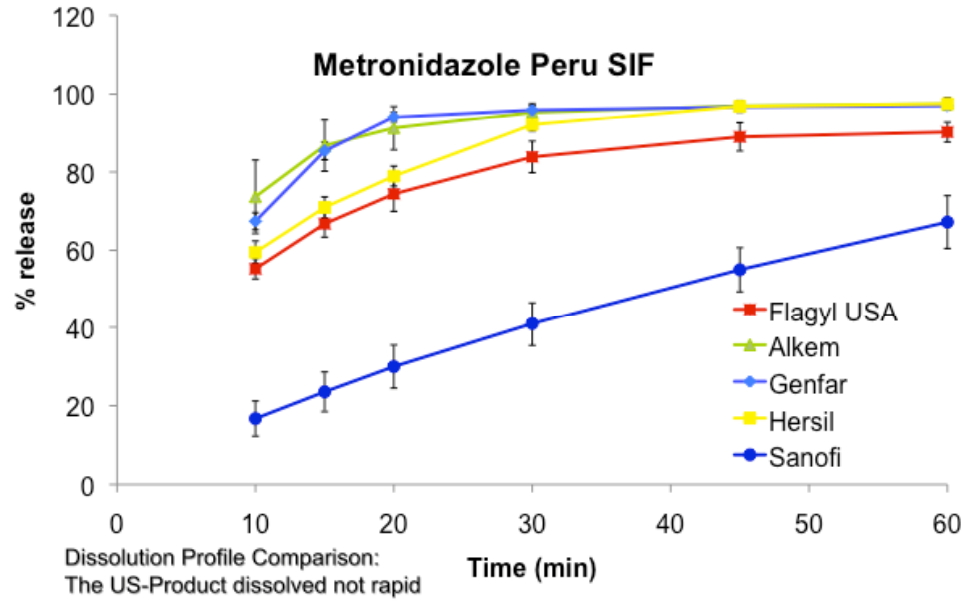
Peru



Dissolution Profile Comparison:
The US-Product dissolved very rapidly.
Genfar and Alkem dissolved very rapidly. Hersil and Sanofi needed up to 45 minut to release > 85% of its content.



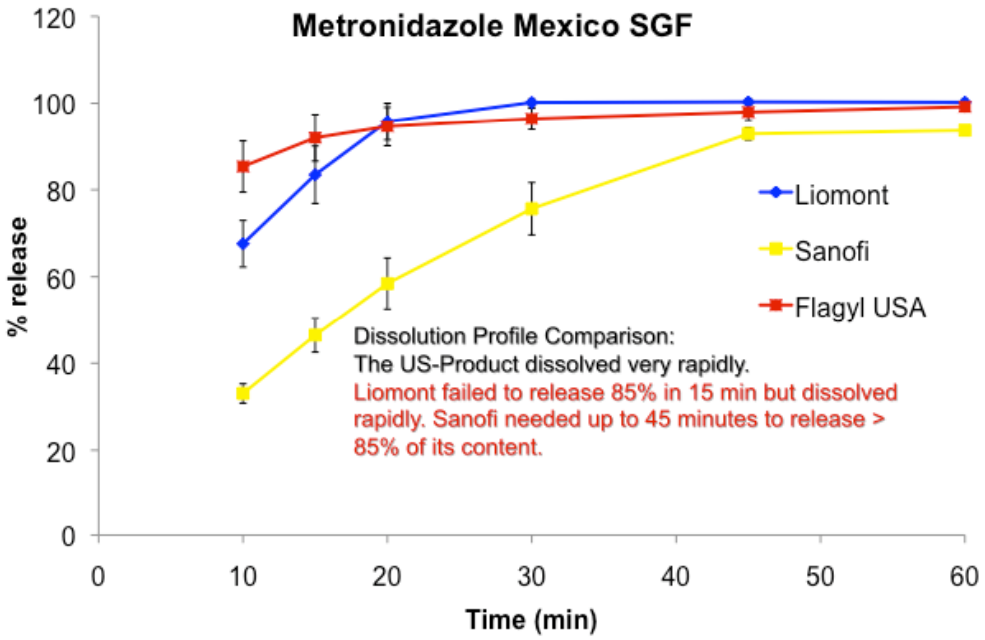
Dissolution Profile Comparison:
The US-Product dissolved rapidly.
Genfar dissolved very rapidly. Hersil has a F2 of 78.9 using the first 30 minutes and Alkem has a F2 of 42.6 (20 min.) when compared to the US product. The Sanofi product needed over 60 min to release 85% of its content; F2= 21.3

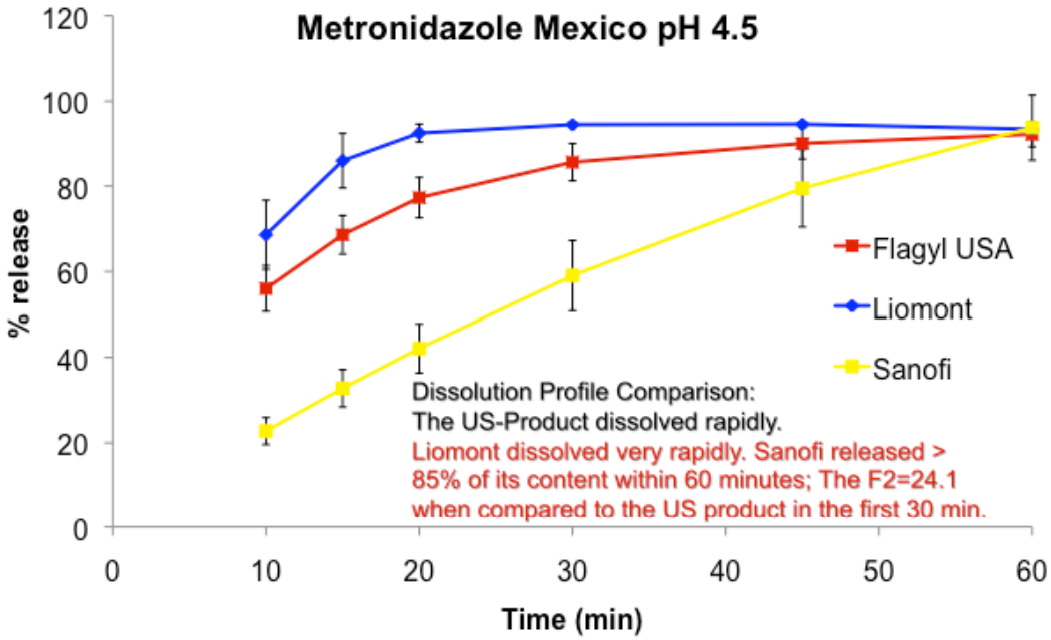


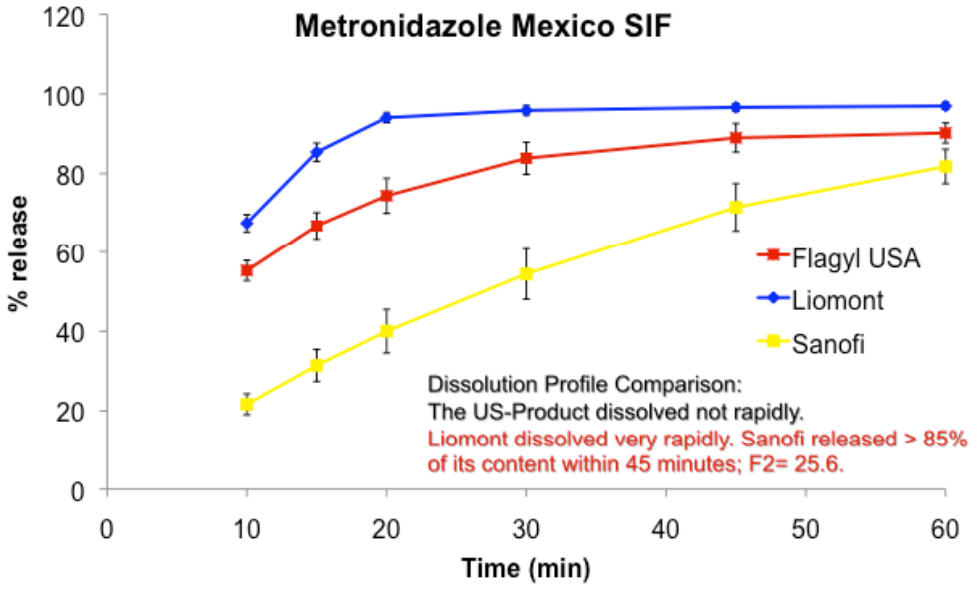
Dissolution Profile Comparison:
The US-Product dissolved not rapid
Genfar and Alkem dissolve very rapidly and can not be compared using F2. Hersil has a F2 of 62.3 and Sanofi a F2 of 18.7 using the first 30 min.

Country	Company	Product	Batch	Exp.	Excipient
USA	Searle Pharmacia	Flagyl 500 mg	C061228	03/09	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
Peru	Hersil	Metronidazol	011017	11/10	Excipients
	Alkem	Metron	7001EA	03/10	Excipients
	Genfar	Metronidazol	020108	01/13	Excipients
	Sanofi Aventis	Flagyl 500 mg	C8R392	01/11	Excipients

Mexico

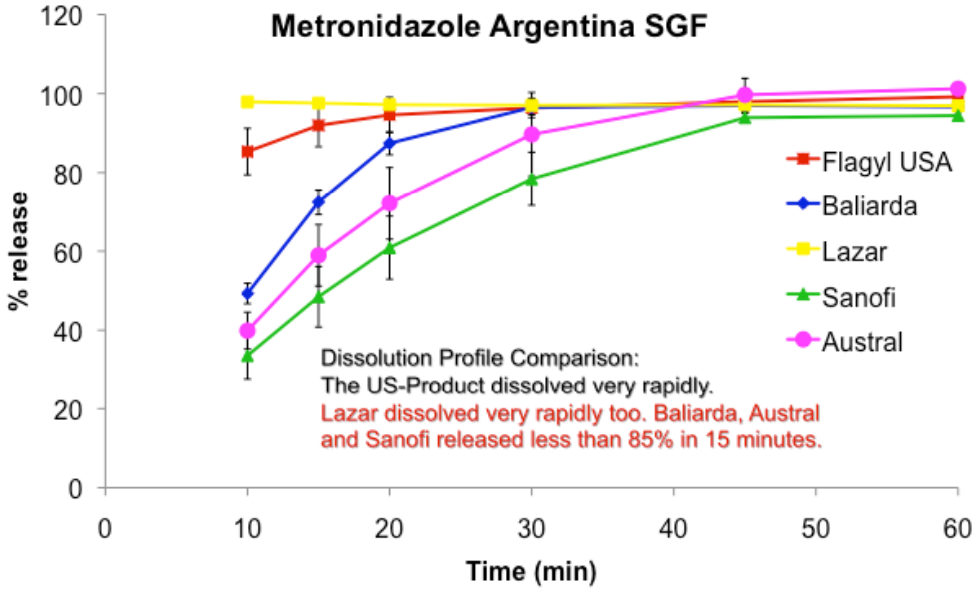


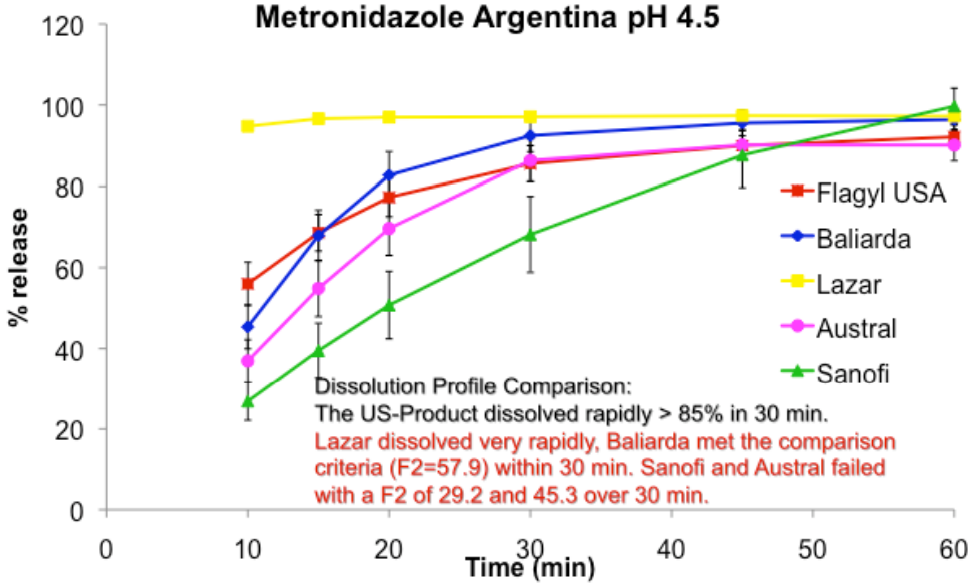


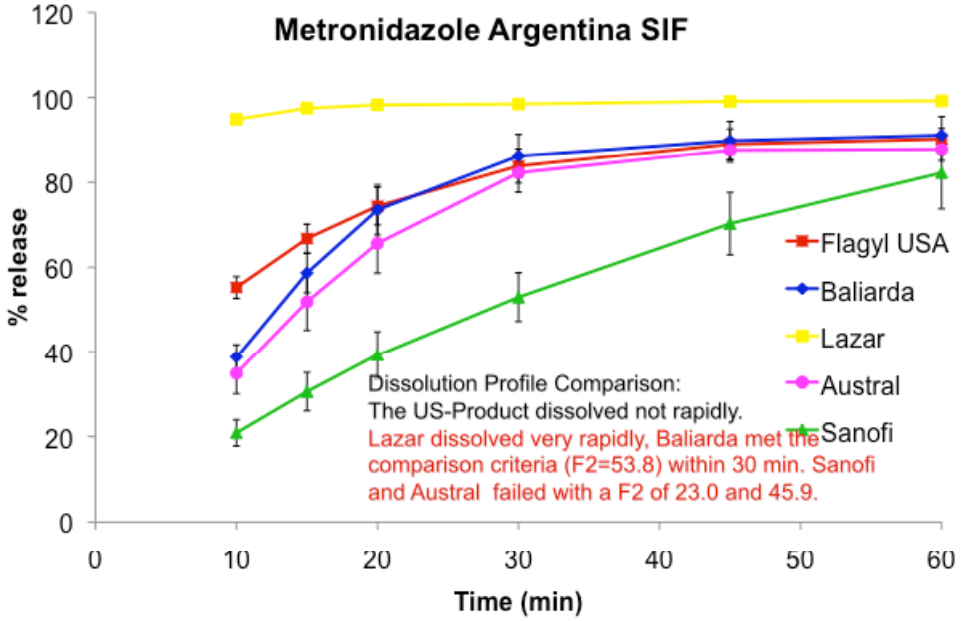


Country	Company	Product	Batch	Exp.	Excipient
USA	Searle Pharmacia	Flagyl 500 mg	C061228	03/09	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
Mexico	Sanofi Aventis	500 mg	B8B575	03/11	Excipients
Mexico	Limont	Flagenase	P07009	07/01	Excipients

Argentina





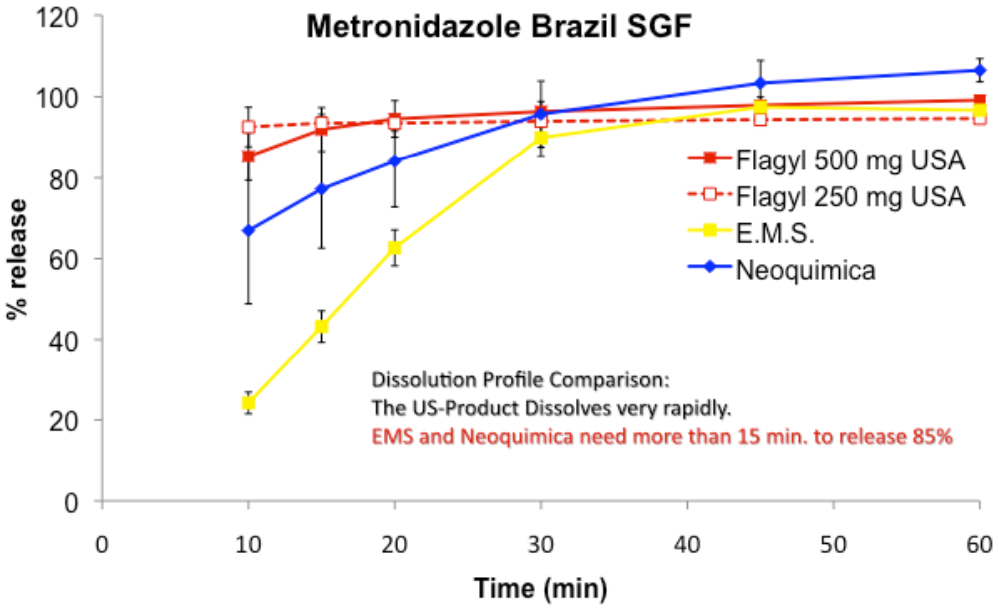


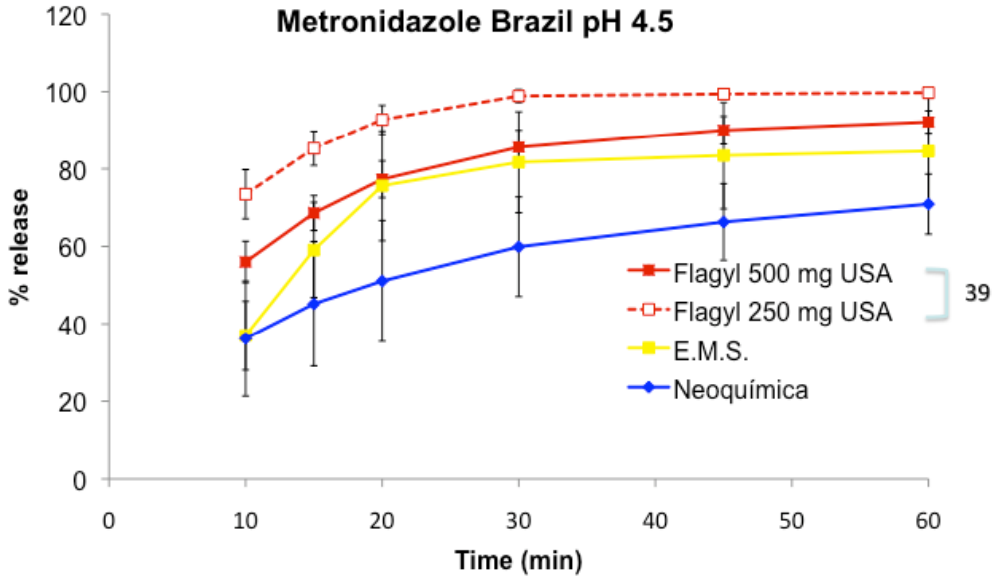
Country	Company	Product	Batch	Exp.	Excipient
USA	Searle Pharmacia	Flagyl 500 mg	C061228	03/09	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
Argentina	Aventis	Flagyl 500 mg	U6121	10/10	Water, Ethanol, Maize Starch, Calcium Phosphate Dihydrate, Mg-stearate, HMPC, Whithe Wax, titanium Dioxide, PEG 20,000 Polyvidone, Sorbitol Anhydrate
	Baliarda	Ginkan	0403	09/10	Maize Starch, Povidon, PEG 6000, Aerosil, AC-DI-SOL, Talcum, Mg-Stearate, HPMC, Propyleneglycol, Titanium Dioxide
	Austral	Metral	L77	02/10	Excipients
	Genfar	Metronidazole	020108	01/13	Excipients

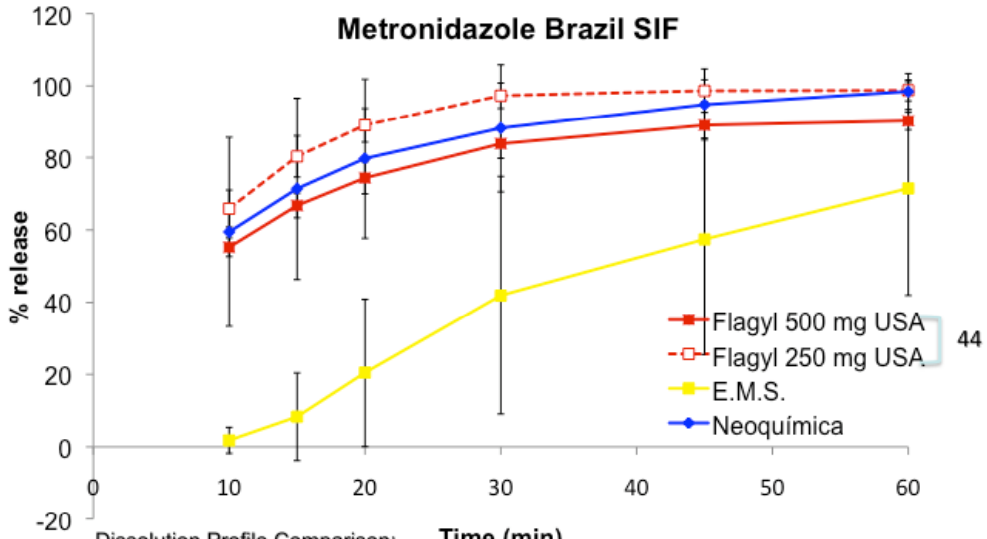
Summary Metronidazole Dissolution comparison vs. Flagyl (US-RLD)

Country	Manufacturer	SGF	pH 4.5	SIF
Peru	Genfar	+	-	-
	Hersil	-	+	+
	Alkem	+		-
	Sanofi	-	-	-
Mexico	Liomont			-
	Sanofi	-	-	-
Argentina	Baliarda	-	-	+
	Lazar	+	-	-
	Sanofi	-	-	-
	Austral	-	-	-

Brazil







Dissolution Profile Comparison:
The 250 mg US-Product dissolved rapidly while the 500 mg dissolved slowly.
Neoquimica and E.M.S. had a SD over 20% and can not be compared to the US product. Some EMS tablets did not disintegrate

Dissolution Profile Comparison:
The 250 mg US-Product dissolved very rapidly while the 500 mg only dissolved rapidly > 85% in 30 min.
Neoquimica and E.M.S. can not be compared with the US product due to high SD in the data.

Country	Company	Product	Batch	Exp.	Excipient
USA	Searle Pharmacia	Flagyl 500 mg	C061228	03/09	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
USA	Pfizer Searle	Flagyl 250	C071099	09/10	Cellulose, Fd&C Blue,Hydroxypropyl Cellulose, Hypromellose, PEG, Stearic Acid, Titanium Dioxide
Brazil	EMS	Metronidazol 400mg	L145675	03/10	Mg-Stearate, Calcium Phosphate, MCC, Povidone, Titanium Dioxide, Macrogol, Methylmethacrylate, Talcum, Croscarmelose Sodium, Hydrogenated Vegetable Oil
	Neo Quimica	Metroidazol 250 mg	82981	01/10	Polyvinylpyroidon, MCC, Mg-Stearate,

Summary Metronidazole Dissolution comparison vs. Flagyl (US-RLD 250/ 500 mg)

Country	Manufacturer	SGF	pH 4.5	SIF
Brazil	Neoquimica (250)	-	-	-
	EMS (400 mg)	-	-	-

Both Products had a too high SD

Metronidazole

- No product is equivalent to the RLD
- 12 products failed *In Vitro* Similarity (IVS)
- The lower and higher dose of the reference product were not *In Vitro* Similar (IVS) in two media.

Conclusions

- The study showed that many BCS class 1 generics are not *in vitro* similar with the the US-RLD or its substitute
- For Flagyl are different innovator products on the world market available
- These products have different biopharmaceutical properties.
- This demonstrates the clear need to make a Global Performance Standard for drugs on the List of Essential Medicines available.
- Pharmaceutical Equivalence together with *In Vitro* Similarity (IVS) are suitable/promising surrogates/parameters to ensure/indicate Therapeutic Equivalence between products

Acknowledgements

- DDIC
  Faculty of Pharmacy
  University of Alberta

- University of Sao Paulo
  Faculty of Pharmaceutical Sciences

- USP

U.S. PHARMACOPEIA