A tiered approach to method validation for the support of a bioequivalence trial with ibuprofen

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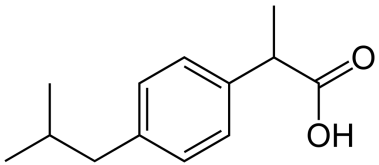
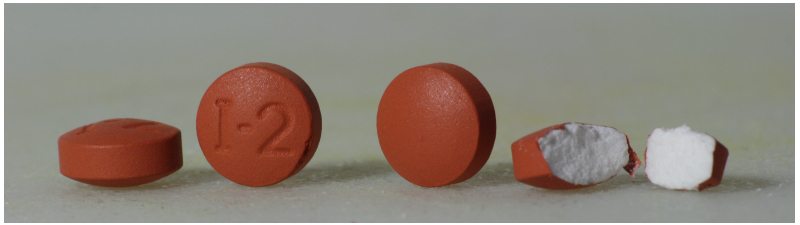
24 November 2021

Ibuprofen



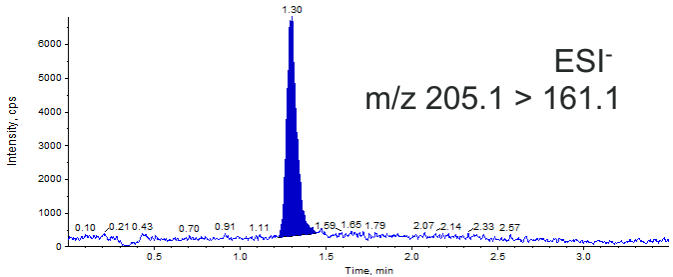
- Discovered in 1961
- Marketed since 1969 for rheumatic diseases
- Approved in 1983 as OTC medicine for mild to moderate pain
- Patent expiry and launching of generic products in 1985
- Currently approved in 82 countries
- Yearly production API: 35,000,000 kg
- Global sales: € 6,000,000,000



Ibuprofen

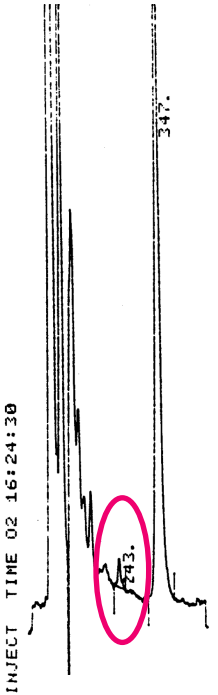
Oral dosing

- 200 to 600 mg, 3 to 4 times per day
- Plasma C_{max} after single oral dose (400 mg): ~ 30 µg/mL
- LLOQ required: high-ng/mL to low-µg/mL level



ESI^-
m/z 205.1 > 161.1

0.25 µg/mL ibuprofen in plasma
PP – LC-MS/MS (2015)

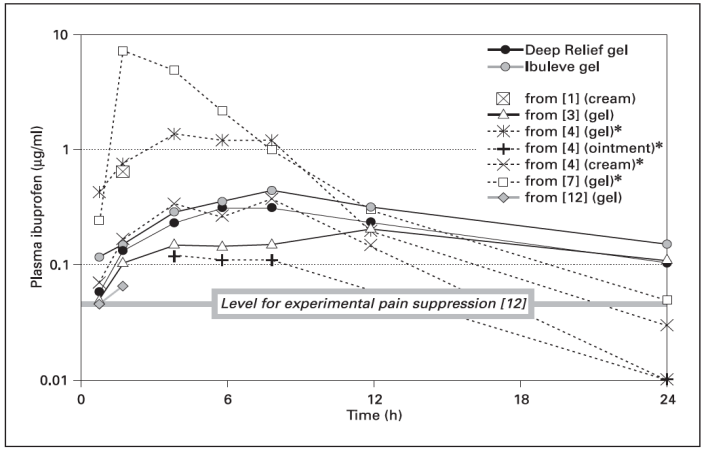


1 µg/mL ibuprofen in plasma
SPE – HPLC-UV_{229} (1984)

Ibuprofen

Topical application

- 5-10% (w/w) gel: 125-500 mg
- Plasma C_{max} after topical dose (400 mg) varies: 0.2 to 7 µg/mL
- Depends on type of gel and occlusion (covering of administration area)
- LLOQ required: low-ng/mL level

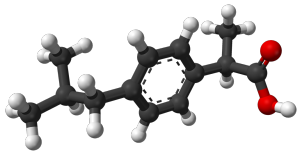


Brain et al, Skin Pharmacol Physiol. 2006;19(1):17-21

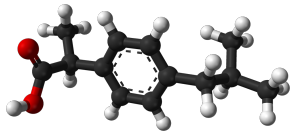
Ibuprofen

Chirality

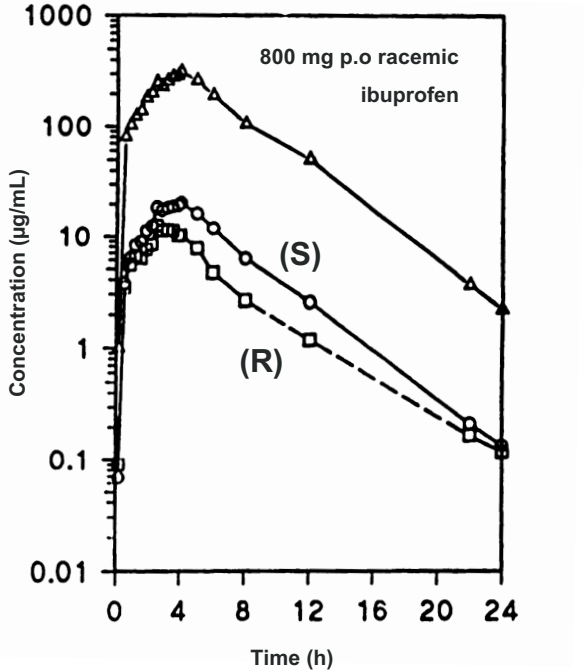
- Ibuprofen is dosed as racemate
- (S)-ibuprofen is active, (R)-ibuprofen is not
- After oral dosing, (R)-ibuprofen is converted to the (S)-form in the gastro-intestinal tract
- Unknown if stereo-conversion also occurs after topical administration



(R)-ibuprofen



(S)-ibuprofen

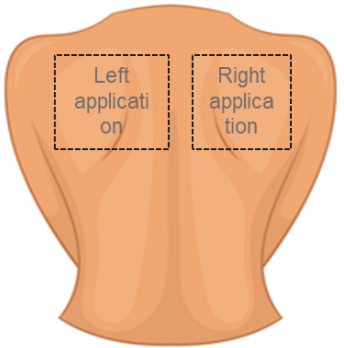


Rudy et al, J. Pharmacol Exp Ther. 1991;259(3):1133-1139

Ibuprofen

Case Study

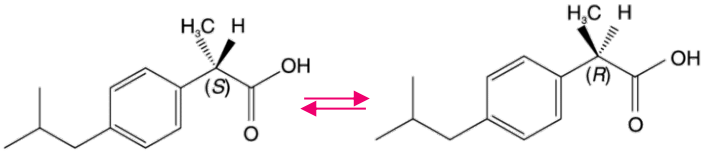
- Bioequivalence trial with 10% (w/w) ibuprofen topical gel
- Administered dose: 125 mg; sampling period: 72 h
- Health authority requirement for separate quantification of (R)- and (S)-enantiomers, because of differences in their pharmacokinetics and pharmacodynamics
- Estimated LLOQ: 0.5 ng/mL per enantiomer
- Chiral method at this level has not been described



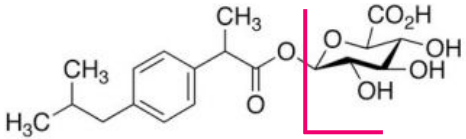
Ibuprofen

Points of attention

- Potential *in vitro* interconversion of enantiomers to be avoided



- Acyl glucuronide metabolite occurs in plasma; *in vitro* back conversion to parent drug to be avoided

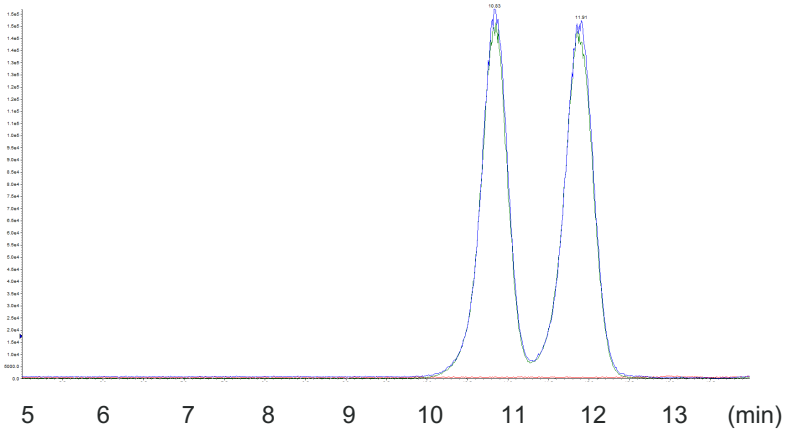


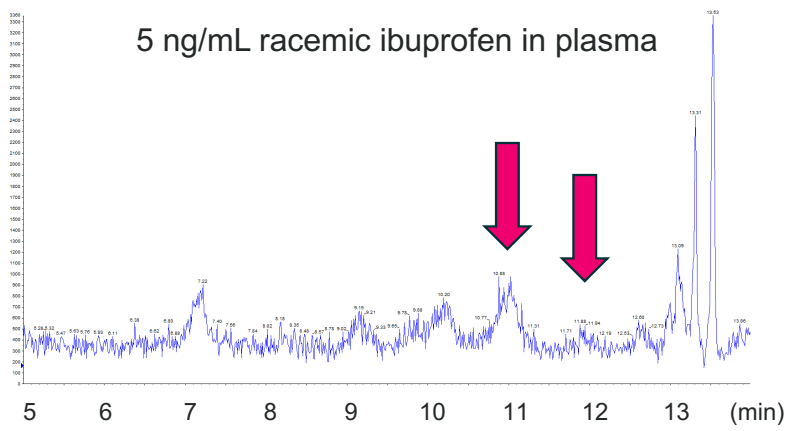
- Both reactions occur at elevated temperature and pH

LC-MS/MS method

Chiral separation

- Long run time (14 min) for ibuprofen enantiomers on chiral column
- Combination of acidic mobile phase, negative ionization and unselective fragmentation (m/z 205.1 > 161.1; loss of CO_2): LLOQ (0.5 ng/mL) not reachable

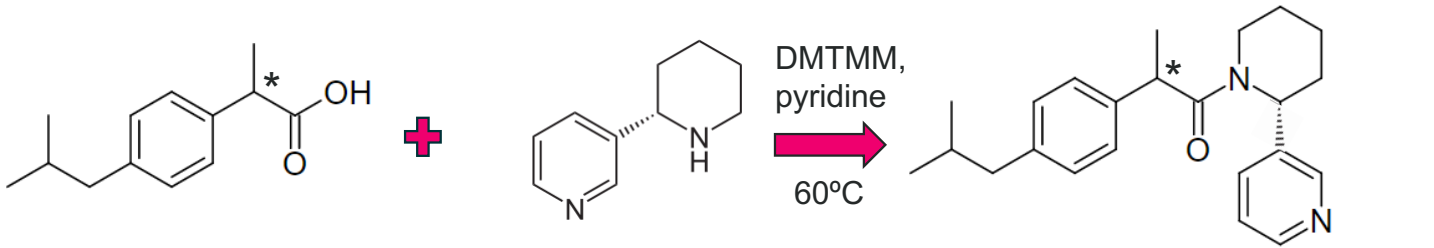




LC-MS/MS method

Chiral separation

- Best approach: derivatization with a chiral reagent containing a well ionizable group to a set of diastereomers
- Separation with standard reversed-phase LC and detection in positive ionization mode

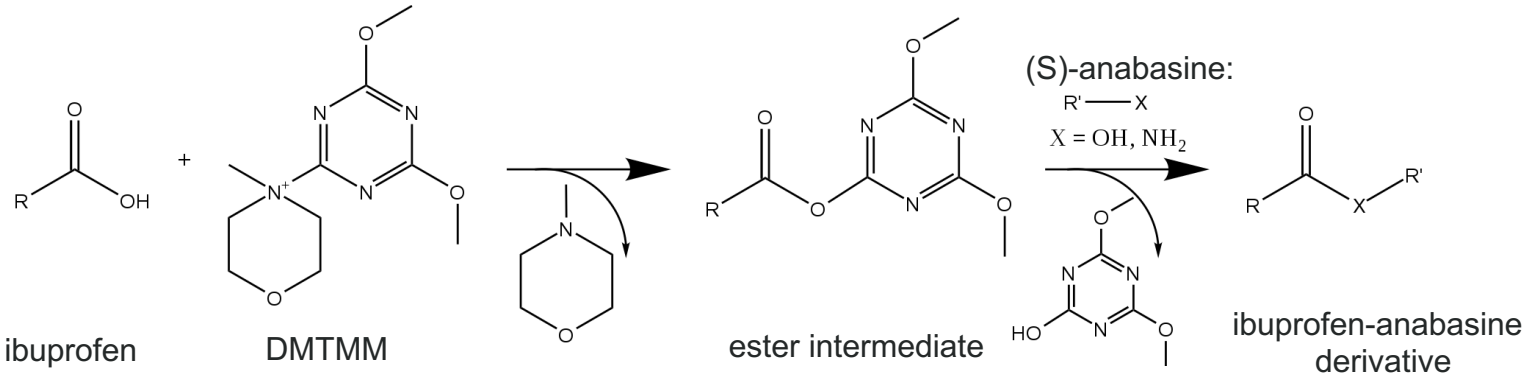


ibuprofen enantiomers (S)-anabasine ibuprofen-anabasine diastereomers

LC-MS/MS method

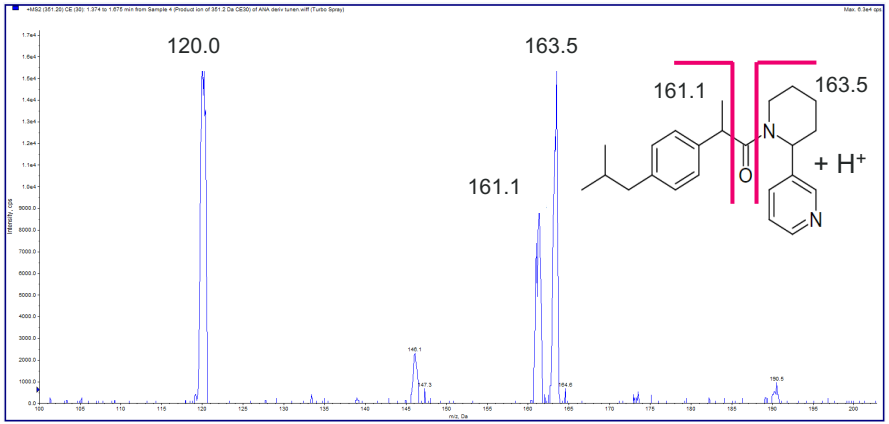
Derivatization

- Activation of carboxylic acid group with DMTMM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride) to a reactive ester
- Further reaction of the ester intermediate with (S)-anabasine to the final amide derivative

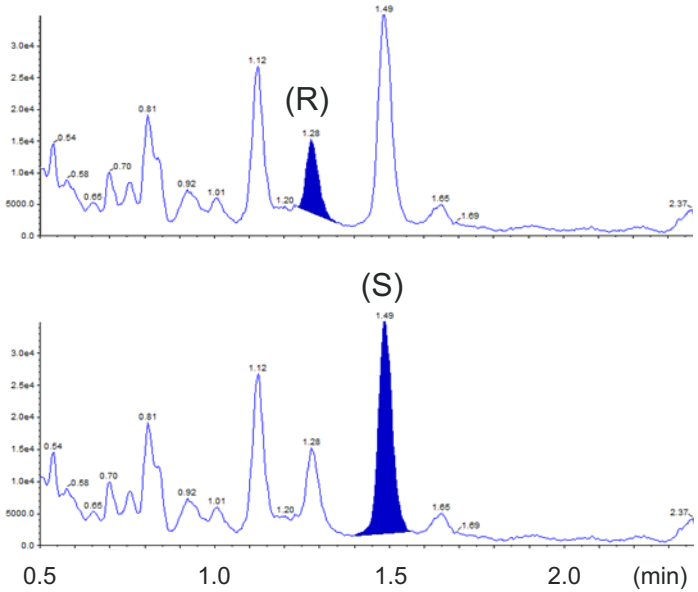


LC-MS/MS method

- Relatively selective mass transition: m/z 351.2 > 161.1
- Method >10x more sensitive and 5x faster than on chiral column



MS/MS spectrum from m/z 351.2



0.5 ng/mL (R)- and (S)-ibuprofen in plasma

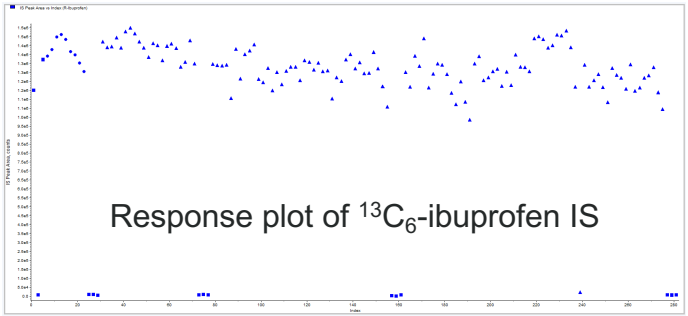
LC-MS/MS method

Instability

- No interconversion between ibuprofen enantiomers during derivatization
- Substantial back-conversion of glucuronide to parent drug during derivatization

Extraction

- 100 µL plasma with 1 mL heptane/TBME (1:1, v/v) at pH 3.5
- High recovery of ibuprofen
- Low recovery of glucuronide
- Removes ibuprofen glucuronide from sample before derivatization



Response plot of $^{13}C_6$-ibuprofen IS

LC-MS/MS method

Selectivity

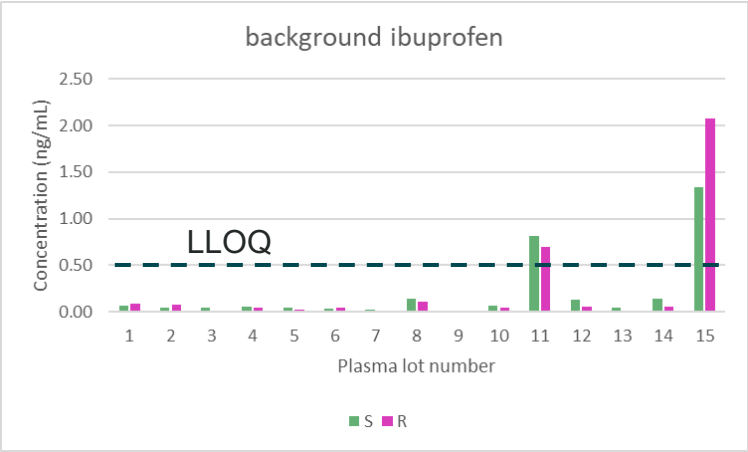
- Background levels of (S)- and (R)-ibuprofen in commercially obtained blank plasma: typically 1- 4000 ng/mL
- Concentrations rarely <20% of LLOQ

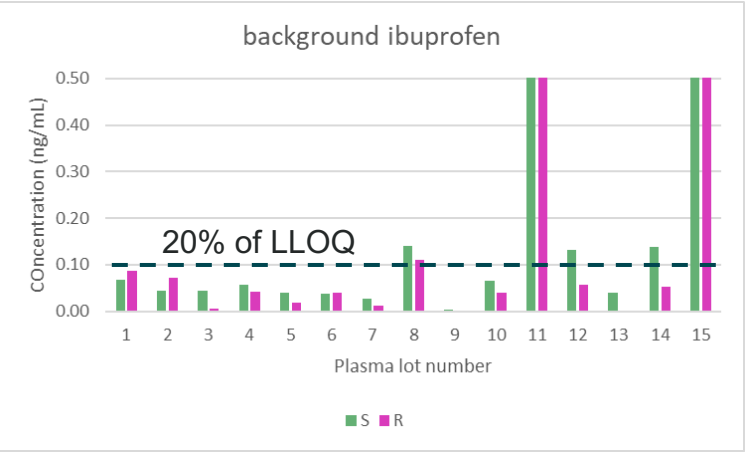
- Due to widespread use and universal occurrence in the environment (e.g. low ng/mL levels in surface water in EU)

LC-MS/MS method

Selectivity

- Detectable background levels of (S)- and (R)-ibuprofen in locally collected blank plasma from volunteers not having taken the drug





Pilot study

- Better estimation of relevant concentration range needed: pilot study with five subjects (tier 1)
- Existing method should reliably determine ibuprofen enantiomer plasma concentrations, preliminary range: 0.5-500 ng/mL
- Method
  - verification
  - qualification
  - abbreviated validation
  - scientific validation
  - fit for purpose validation

Pilot study

Parameter	Done	Max acceptance limits	Note
Enantiomer identification	✓		Covered during MD
Calibration	✓	20% bias/CV (25% at LLOQ)	
Accuracy and precision	✓ (one run)	20% bias/CV (25% at LLOQ)	
Integrity of dilution	-		
Selectivity	✓	25% of LLOQ	Covered during MD
Matrix effect	-		
Recovery	-		
Autosampler stability	-		
Bench-top stability	✓ (ice)	20% bias/CV	
	✓ (RT)	20% bias/CV	Covered during MD
Freeze-thaw stability	✓ (-20ºC to RT)	20% bias/CV	Covered during MD
Blood stability	✓ (ice, RT)	20% bias/CV	Covered during MD
Frozen stability	✓ (-20ºC)	20% bias/CV	
Stock stability	✓ (RT, 4ºC)	5% bias/10%CV	

Pilot study

Stability assessments with

- ibuprofen racemate (low and high level)
- (R)-/(S)-ibuprofen ratio of 1:10 and 10:1 to study interconversion (high level)
- ibuprofen racemate plus equimolar amount of (racemic) ibuprofen glucuronide to study back-conversion (high level)

Pilot study

Plasma	(R)-ibuprofen Bias from nominal (%)		(S)-ibuprofen Bias from nominal (%)	
Ibuprofen racemate	Low	High	Low	High
T=0	-4.1	-6.3	-6.6	-6.3
20 h RT	-3.4	-6.9	-10.3	-4.6
24 h ice	+0.7	+1.9	+18.3	-1.6
5 F/T cycles	-5.9	-4.8	-9.8	-4.1
24 days -20ºC	+1.0	+3.1	+3.5	-0.8
Excess (R)-ibuprofen	High		High	
T=0	-13.8		+2.2	
20 h RT	-10.6		+0.7	
24 h ice	+4.3		+7.4	
5 F/T cycles	-11.3		+1.8	
24 days -20ºC	+4.4		+8.6	
Excess (S)-ibuprofen	High		High	
T=0	-5.5		-13.4	
20 h RT	-5.9		-14.9	
24 h ice	+12.6		+3.4	
5 F/T cycles	-4.4		-13.4	
24 days -20ºC	+13.6		+1.5	
Plus ibuprofen glucuronide	High		High	
T=0	+1.7		+4.5	
20 h RT	+22.5		+28.4	
24 h ice	+6.0		+8.0	
5 F/T cycles	+1.8		+4.7	
24 days -20ºC	+2.4			

No conversion of (10-fold excess) of other enantiomer

Some back-conversion of glucuronide at RT

Pilot study

Whole blood	(R)-ibuprofen Bias from nominal (%)		(S)-ibuprofen Bias from nominal (%)	
Ibuprofen racemate T=0 2 h ice 2 h RT	Low ref +1.7 +2.8	High ref +1.6 +1.6	Low ref -1.4 -1.9	High ref +0.8 +1.3
Excess (R)-ibuprofen T=0 2 h ice 2 h RT	High ref -1.8 +0.8		High ref -18.3 -8.1	
Excess (S)-ibuprofen T=0 2 h ice 2 h RT	High ref +5.2 -12.7		High ref -0.4 0.0	
Plus ibuprofen glucuronide T=0 2 h ice	High ref +3.0		High ref +0.3	

No conversion of (10-fold excess) of other enantiomer

No back-conversion of glucuronide on ice

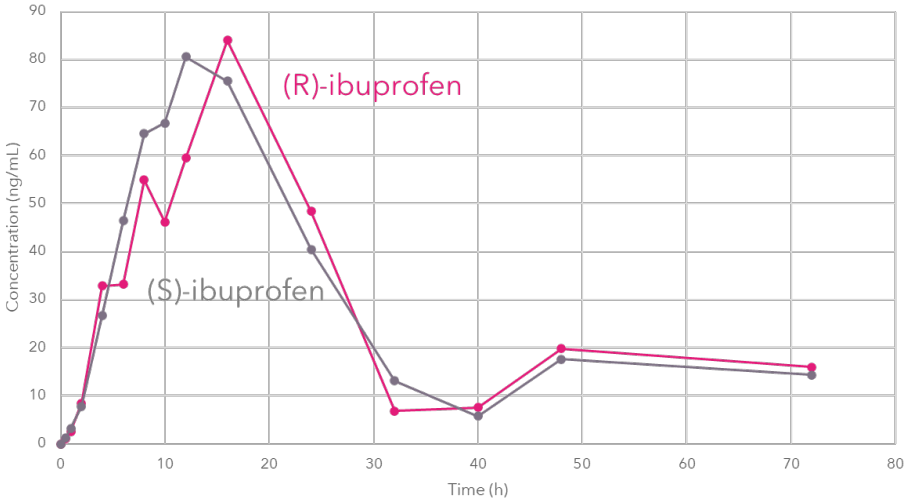
Pilot study

Conclusions

- Regular blood sampling (storage on ice, plasma harvesting within 1 h) is OK
- Plasma storage on ice to prevent any back-conversion of glucuronide
- Positive pre-dose samples to be expected

Pilot study

- Sample analysis: 4/6/20
- 80% of the subjects had quantifiable (S)- and (R)-ibuprofen in pre-dose samples (1-10 ng/mL) in both periods
- C_{max}: 40-200 ng/mL
- No PK difference for (S)- and (R)-ibuprofen



Bioequivalence study

- BE study with 38 subjects (tier 2)
- Additional blood sampling point
- Calibration range of preliminary method adapted to reflect actual *in vivo* drug levels range: 0.5-300 ng/mL
- Full method validation across new range
  - 15 blank plasma lots for selectivity
  - Stability also for 10:1 and 1:10 ratio of enantiomers
  - Stability also in presence of glucuronide metabolite

Bioequivalence study

- Sample analysis: 4/6/15
- Including ISR
- 50% of the subjects had quantifiable (S)- and (R)-ibuprofen in pre-dose samples (0.5-10 ng/mL) in both periods
- C_{max}: 30-200 ng/mL
- No PK difference for (S)- and (R)-ibuprofen

Conclusions

- A tiered bioanalytical approach was followed to support a BE trial with topical ibuprofen
- Tier 1: a limited, science-based validation of a preliminary chiral method across an estimated calibration range, and exploratory analysis of samples from a pilot study
- Tier 2: a full regulatory validation of the final method across an adapted calibration range, and analysis of samples from the actual BE study

Thank you!

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