IVRT Method Development for API Suspension Products and Validation with In Vivo Models

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Session Description and Objectives

- Presentation of *in vitro* release testing method development for LAI parenteral suspensions

- To develop *in vitro* release testing methods that better correlate with *in vivo* release
- Especially develop longer tests.
- Compare various testing methods.
- Develop IVIVCs.

What are Long-acting Injectable (LAI) Suspensions?

- **Long-acting:** extended release for a period from 1 week to several months.
- **Injectable:** can be injected *via* intramuscularly (IM), subcutaneously (SC) and local areas (*e.g.*, intra-articular)
- **Suspensions:** can be "for suspension" or "suspension".

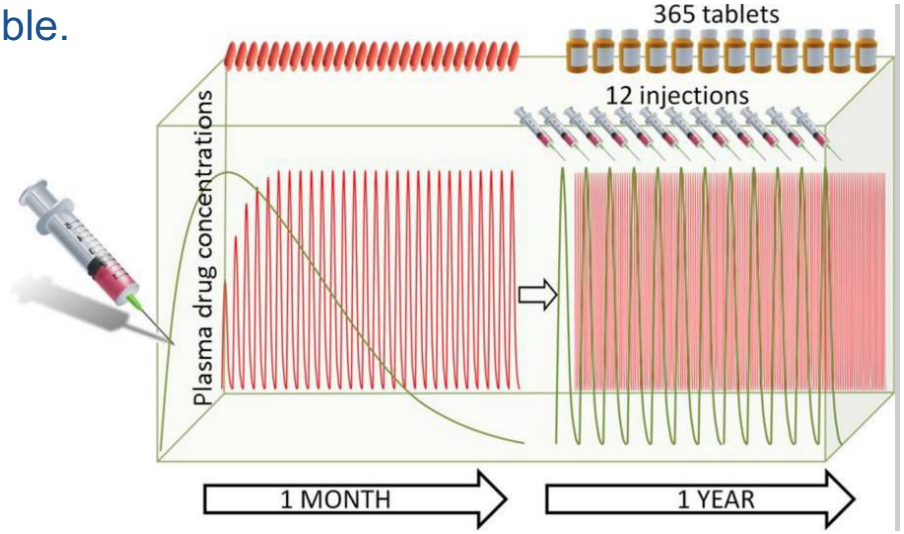
i. "For suspension": stored in powder and reconstituted using appropriate diluent prior to use and dosed as suspension, such as microspheres and drug powders.

ii. Suspension: can be in the form of oil or aqueous based drug crystalline suspensions.

- **Disease area:** chronic diseases/conditions such as schizophrenia, diabetes, contraception, inflammation or pain control, cancers, HIV, etc.

Advantages of LAI Formulations

- Extended drug release.
- Flip-flop pharmacokinetics, where the apparent half-life is controlled by the absorption rate constant.
- Predictable correlation between dosage and plasma levels.
- *In vitro-in vivo* correlation (IVIVC) is possible.
- Reduced peak-trough plasma levels
- Consistent bioavailability
- Good patient adherence
- Reduced pill burden



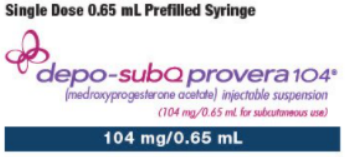
Mechanisms of Long-Acting Effect of LAIs

Slow drug dissolution: crystal drug suspension

Drug power in vial

Drug power in a prefilled dual chamber syringe

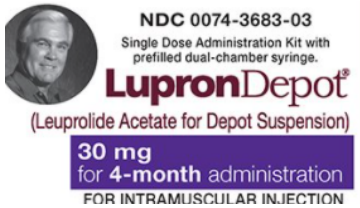
Drug suspension in a prefilled syringe



Drug suspension in vial

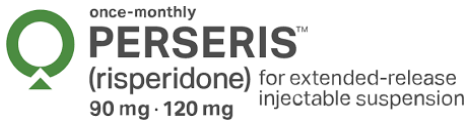
PLGA based: microspheres; *in situ* forming implants

Microsphere powder in a prefilled dual chamber syringe



In situ implants: ATRIGEL® delivery system

Microsphere powder in



- Slow dissolution due to the extremely low water solubility of the drug: aqueous crystalline suspensions
- Polymer controlled drug release such as poly(D,L-lactide-co-glycolide) (PLGA): microspheres and *in situ* forming implants.

U.S. FDA Approved LAI Aqueous Suspensions

Active Ingredient	Proprietary Name	Route	Applicant Holder	Treatment	Approval Date	Efficacy duration	Note
Aripiprazole	Abilify Maintena Kit	IM	Otsuka	Schizophrenia	2013	1 month	Powder for suspension
					2014	1 month	
Aripiprazole Lauroxil	Aristada	IM	Alkermes	Schizophrenia	2015	1 month	Ready-to-use suspension
					2017	2 months	
	Aristada Initio Kit	IM	Alkermes	Schizophrenia	2018	1 month	Ready-to-use nanosuspension
Medroxyprogesterone Acetate	Depo-Provera	IM	Pfizer	Contraception	Prior To 1982	3 months	Ready-to-use suspension
					1992	3 months	
	Depo-SubQ Provera 104	SC	Pfizer	Contraception	2004	3 months	
Olanzapine Pamoate	Zyprexa Relprevv	IM	Eli Lilly	Schizophrenia	2009	2-4 weeks	Powder for suspension
Paliperidone Palmitate	Invega Trinza	IM	Janssen	Schizophrenia	2015	3 months	Ready-to-use suspension
	Invega Sustenna	IM	Janssen	Schizophrenia	2009	1 month	Ready-to-use nanosuspension

- In total, 8 LAI aqueous suspension products have been approved by the U.S. FDA.
- Most of the formulations are micro suspensions (with a particle size > 1 μm). There are two nanosuspensions (Aristada Initio Kit and Invega Sustenna) in the form of nanosized drug crystals.

FDA Recommended Dissolution Methods for LAI Suspensions

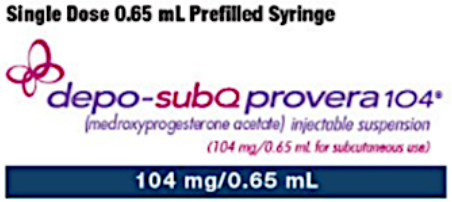
				Dissolution methods			
Active Ingredient	**Proprietary Name**	**Approval Date**	**Efficacy duration**	**Dissolution Apparatus**	**Speed**	**Media/Volume**	**Sampling time (minutes)**
Aripiprazole	Abilify Maintena Kit	2013	1 month	**USP II** (paddle)	50 rpm	0.25% SDS solution /900 mL	10, 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480
		2014	1 month				
Aripiprazole Lauroxil	Aristada	2015	1 month				
		2017	2 months				
	Aristada Initio Kit	2018	1 month				
Medroxyprogesterone Acetate	Depo-Provera	Prior To 1982	3 months	**Test 1**: **USP IV** (flow through cell), 22.6 mm cells, 13 g of 1 mm beads; **Test 2: USP II** (paddle); (provide both data)	**Test 1**: 17 mL/min; **Test 2**: 50 rpm	**Test 1**: 0.5% SDS water /open mode; **Test 2**: 0.35% SDS water /900 mL	**Test 1**: 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90; **Test 2**: 5, 10, 15, 30, 60, 90, 120, 240, 360, 1440 and 2880
		1992	3 months				
	Depo-SubQ Provera 104	2004	3 months				
Olanzapine Pamoate	Zyprexa Relprevv	2009	2-4 weeks	**USP IV** (flow through cell), 22.6 mm cell	3 mL/min	1% SDS in pH 6.8 phosphate buffer /open mode	10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, 360, 480, 600, and 720
Paliperidone Palmitate	Invega Trinza	2015	3 months	**USP II**	50 rpm	0.498% polysorbate 20 in 0.001 N HCl @ 25° C /900 mL	1.5, 5, 8, 10, 15, 20, 30 and 45
	Invega Sustenna	2009	1 month				5, 30, 60, 90, 120, 180, 240, 300 and 360

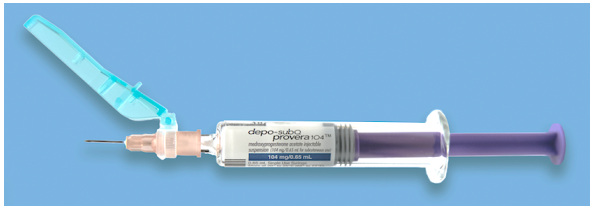
- The duration of the recommended dissolution methods ranges from 45 min to 2880 min (two days).
- these methods may be impractical to establish IVIVCs due to their much shorter release duration when compared to the product *in vivo* performance (*e.g.*, efficacy duration) which ranges from weeks to months in the clinical setting.

Reference Listed Drug (RLD): Depo-SubQ Provera 104®

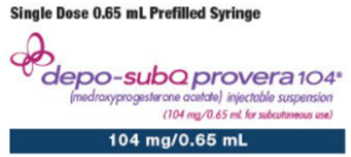
- **Composition Table**

Component	Amount	Function
Medroxyprogesterone acetate	104 mg	Active pharmaceutical ingredient
Methylparaben	1.040 mg	Preservative
Propylparaben	0.098 mg	Preservative
Sodium Chloride	5.200 mg	Tonicity adjusting agent
Polyethylene Glycol	18.688 mg	Suspending agent
Polysorbate 80	1.950 mg	Surfactant/wetting agent
Monobasic Sodium Phosphate . H_2O	0.451 mg	Buffering agent
Dibasic Sodium Phosphate . $12H_2O$	0.382 mg	Buffering agent
Methionine	0.975 mg	pH stabilizing agent
Povidone	3.250 mg	Suspending agent
Water for Injection	q.s.	External media





Preparation of Q1/Q2 Equivalent LAI MPA Suspensions



- **Formulation parameters**
  Particle size and size distribution
- **Preparation**

1) recrystallization of API: anti-solvent method.

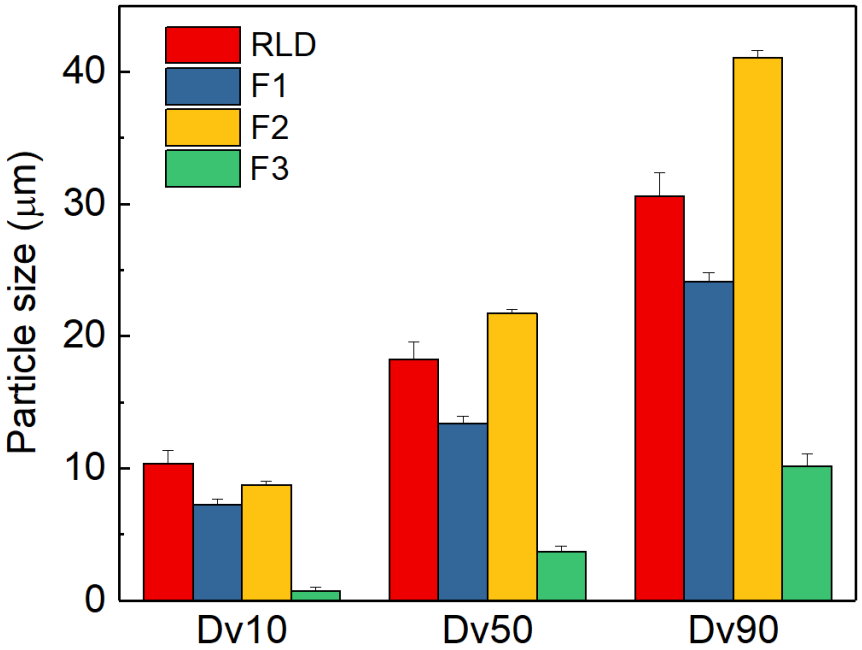
2) preparation of Q1/Q2 equivalent LAI suspensions with different particle size: API dispersed (magnetic stirring) in suspending media (prepared in advance) based on the formula of the RLD.

Formulation	API used
F1	As received
F2	Recrystallized using antisolvent method (acetone:water=1:1)
F3	Probe sonication for 5 minutes

- **Characterization**

Drug content and uniformity; pH; drug solubility in suspension; solid state characterization (PXRD, DSC and TGA); particle size and size distribution; morphology.

Particle Size of the LAI MPA Suspensions



- Particle size and size distribution of RLD and the prepared MPA suspensions. (All data are presented as mean ± SD, n=3)

- The mean particle size of the formulations followed the rank order of F3<F1<RLD<F2.

IVRT Method Development: USP Apparatus 2 with Dialysis Sacs

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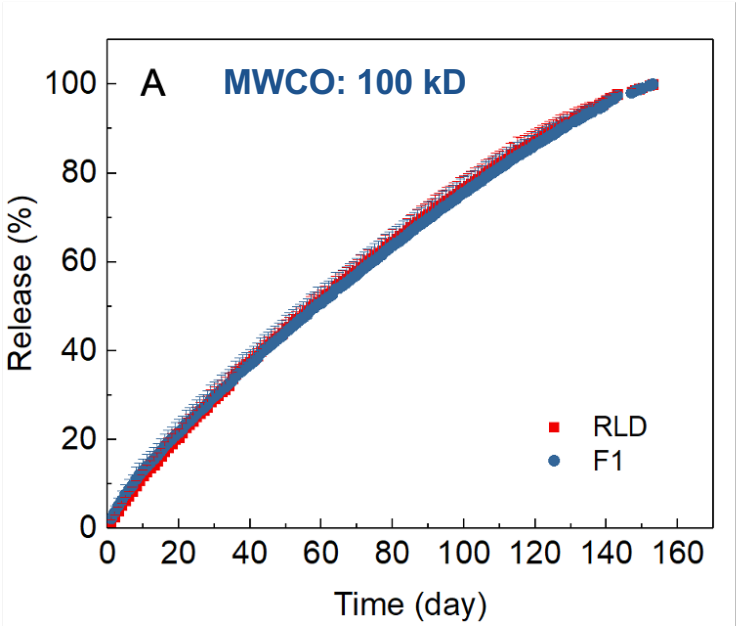
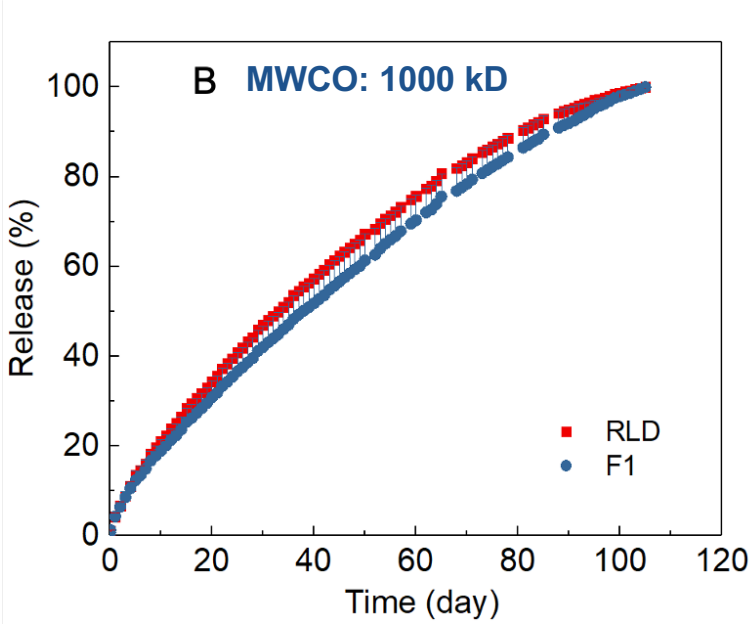
- **Dialysis sac method:** Float-A-Lyzer G2 1 mL, two molecular weight cutoffs (100 kD and 1000 kD)

- Setup of USP apparatus 2 (Sotax AT Extend) with 1 mL Float-A-Lyzers (the accessories used to secure the Float-A-Lyzers were produced in-house) in 300 mL mini-vessels.
- Release media: 300 mL pH 7.4 PBS containing 1% w/v SDS at 37° C.
- Speed: 100 rpm.
- Sample loading: 100 µL of MPA suspension was mixed with 900 µL of pH 7.4 PBS.

IVRT Method Development: USP Apparatus 2 with Dialysis Sac Method





- *In vitro* release profiles of the RLD and F1 using the USP apparatus 2 with Float-A-Lyzers with different MWCOs: **A) 100 kD;** and **B) 1000 kD**. The *in vitro* release testing was conducted in 300 mL of 1% w/v SDS in pH 7.4 PBS at 37° C with a rotation speed of 100 rpm (mean ± SD, n=3).

- Float-A-Lyzer method had low sensitivity in differentiating the RLD and F1 despite the Float-A-Lyzer with the higher MWCO (1000 kD) being slightly better.
- This low sensitivity in differentiating the formulations may be due to violation of sink conditions in the dialysis sacs.

IVRT Method Development: USP App 2 with Enhancer Cells

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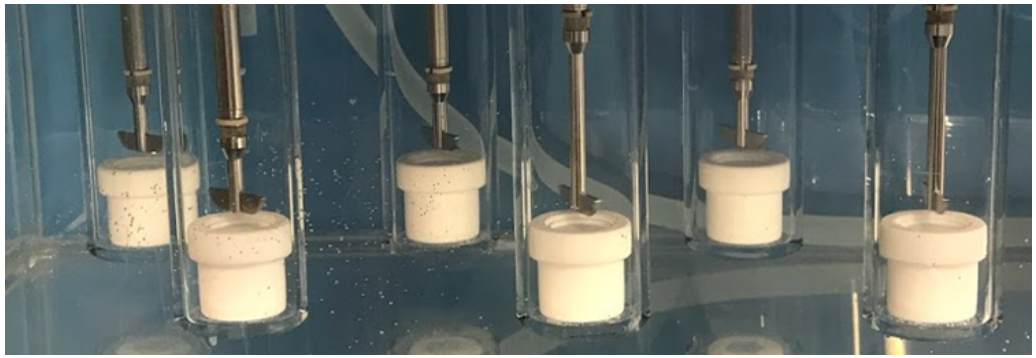
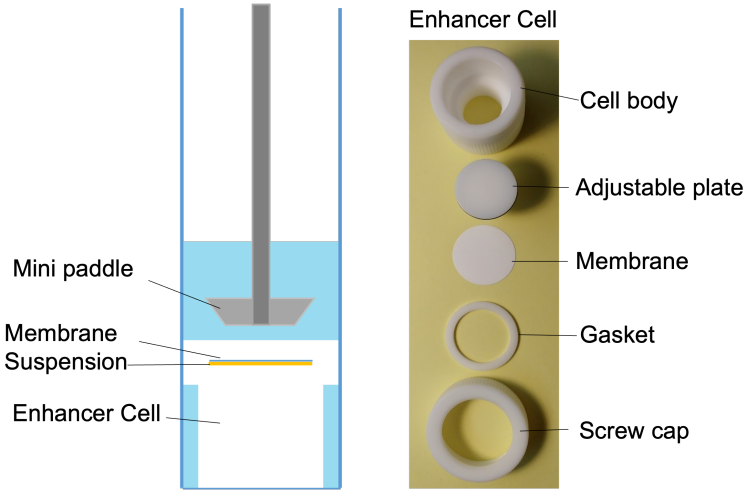
❑ Enhancer cell method





- Instrument: USP apparatus 2 (Sotax AT7 bath) with 200 mL flat-bottomed vessels and mini paddles.
- Enhancer cells with contact area of 4 cm^2.
- Release media: 150 mL pH 7.4 PBS containing 1% w/v SDS at 37° C.
- Speed: 120 rpm.
- Sample loading: 50 µL of the MPA suspension was placed in the center of the enhancer cell compartment.
- Membrane: Whatman ® GF/D, pore size 2.7 µm fiber glass filter.

IVRT Method Development: USP App 2 with Enhancer Cells

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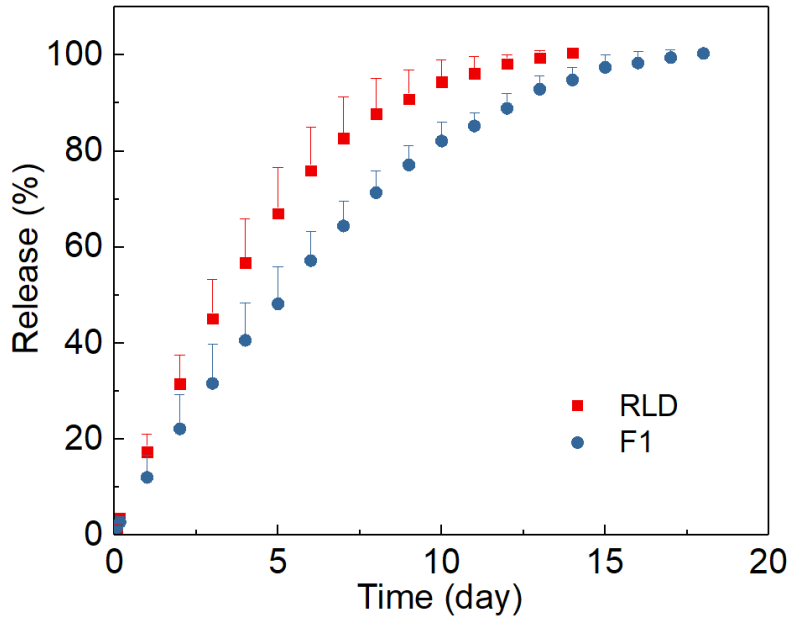
Enhancer cell method



- *In vitro* release profiles of the RLD and F1 using the USP apparatus 2 with enhancer cells with a membrane pore size of 2.7 µm). Release testing was performed in 150 mL of 1% w/v SDS in pH 7.4 PBS at 120 rpm and 37° C (mean ± SD, n=3).

- This method was able to differentiate the RLD and F1 with acceptable reproducibility.
- Release duration was approximately two weeks.

IVRT Method Development: USP App 2 with In-House Designed Adapters

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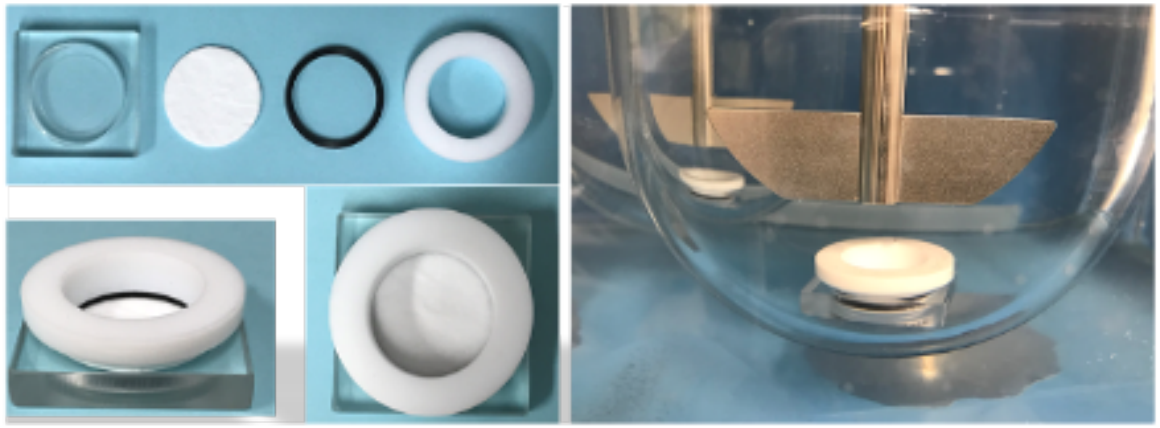
In-house designed adapter method



- Setup of USP apparatus 2 (Sotax AT7 bath) with standard 1-liter vessels and paddles.
- In-house designed adapter with a contact area of 3.14 cm^2.
- Release media: 500 mL water containing 1% w/v SDS at 37° C.
- Speed: 100 rpm.
- Sample loading: 50 µL of MPA suspension was loaded in the center of the sampling compartment of the designed adapters.
- Membrane: Whatman® GF/D, pore size 2.7 µm fiber glass filter.

IVRT Method Development: USP App 2 with In-House Designed Adapters

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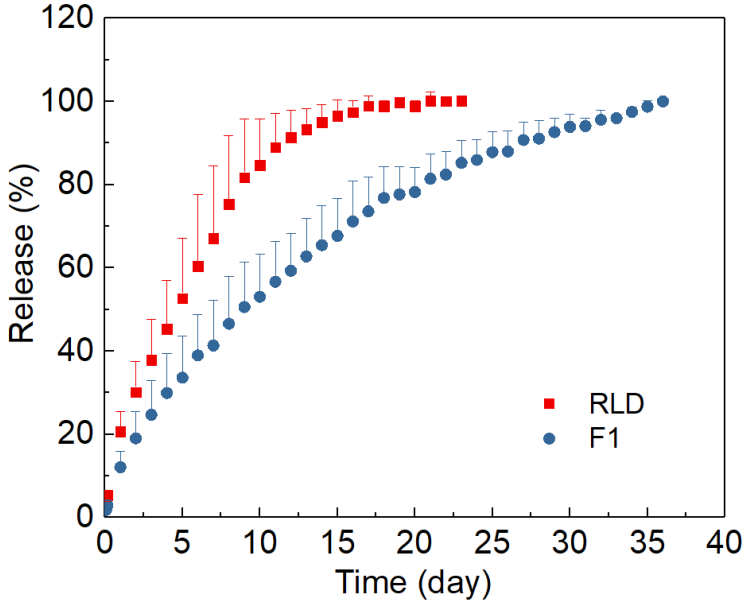
In-house designed adapter method



- *In vitro* release profiles of the RLD and F1 using the USP apparatus 2 with in-house designed adapters with a membrane pore size of 2.7 µm. The release testing was performed in 500 mL of 1% w/v SDS in water at 100 rpm and 37° C (mean ± SD, n=3).

- The release duration of both formulations was longer (~1 month) than with the enhancer cell method (~2 weeks).
- Good discriminatory capability despite the high error bars.
- The adapters need further manufacturing design optimization.

IVRT Method Development: USP App 4 with Semisolid Adapters

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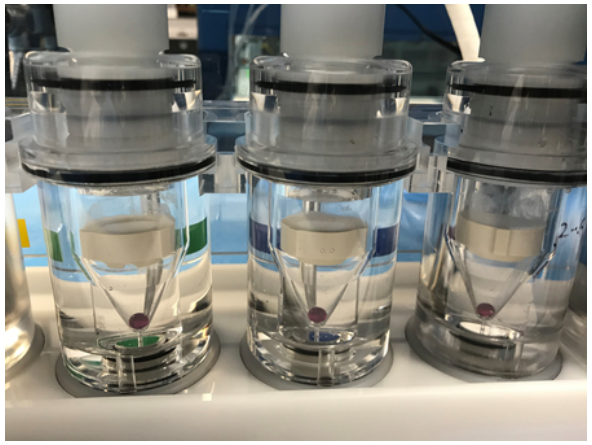
❑ Semisolid Adapter method



- Setup of USP apparatus 4 (Sotax CE7 with CP7-35 piston pump) with flow cells of 22.6 mm diameter.
- Semisolid adapters: 1 mm in depth, without membrane.
- Release media: 500 mL water containing 1% w/v SDS at 37° C.
- Flow rate: 2 mL/min, 4 mL/min and 8 mL/min.
- Sample loading: 50 µL of MPA suspension was loaded in the center of the adapters.
- Two fiber glass filters (Whatman® GF/D (pore size: 2.7 µm) and GF/F (pore size: 0.7 µm)) were used in the filter head of the flow cells.

IVRT Method Development: USP App 4 with Semisolid Adapters

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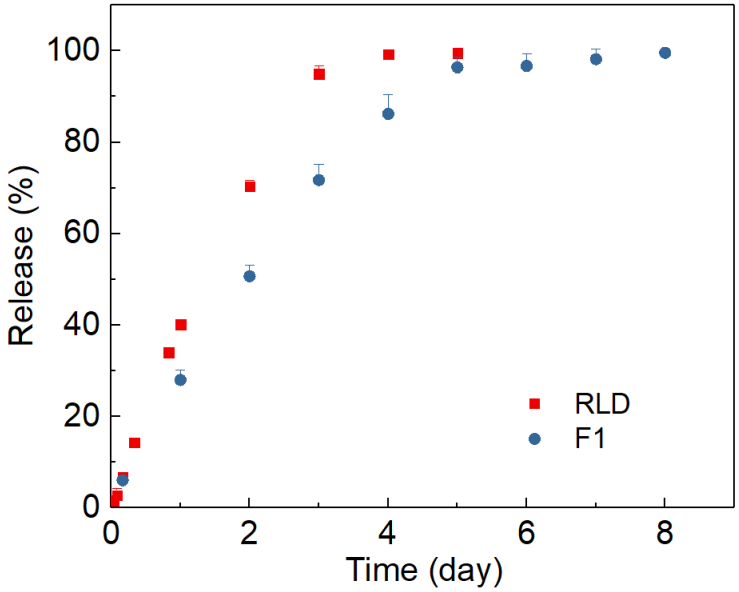
❑ Semisolid adapter method



- *In vitro* release profiles of the RLD and F1 using the USP apparatus 4 with semisolid adapters. Release testing was performed in 500 mL of 1% w/v SDS in water at a flow rate of 8 mL/min and 37° C (mean ± SD, n=3).

- The release duration for both the RLD and F1 was approximately 1 week.
- The developed USP apparatus 4 with the semisolid adapter method showed good discriminatory capability with good reproducibility (less than 5% RSD) for the RLD and F1.

IVRT Method Development: USP App 4 with Semisolid Adapters

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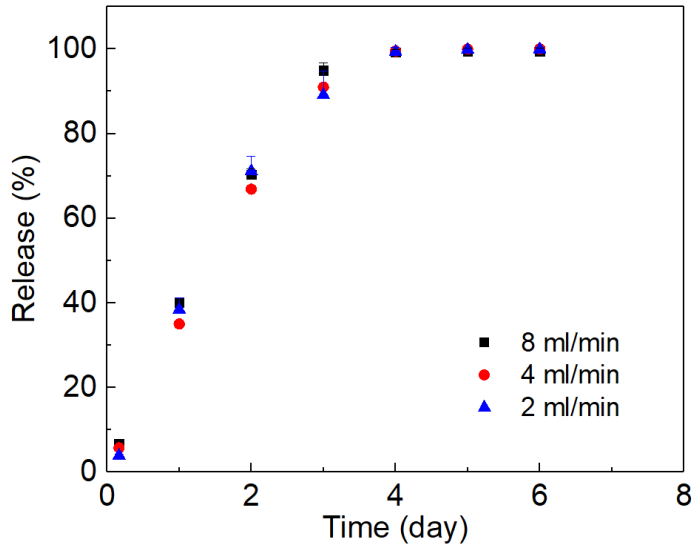
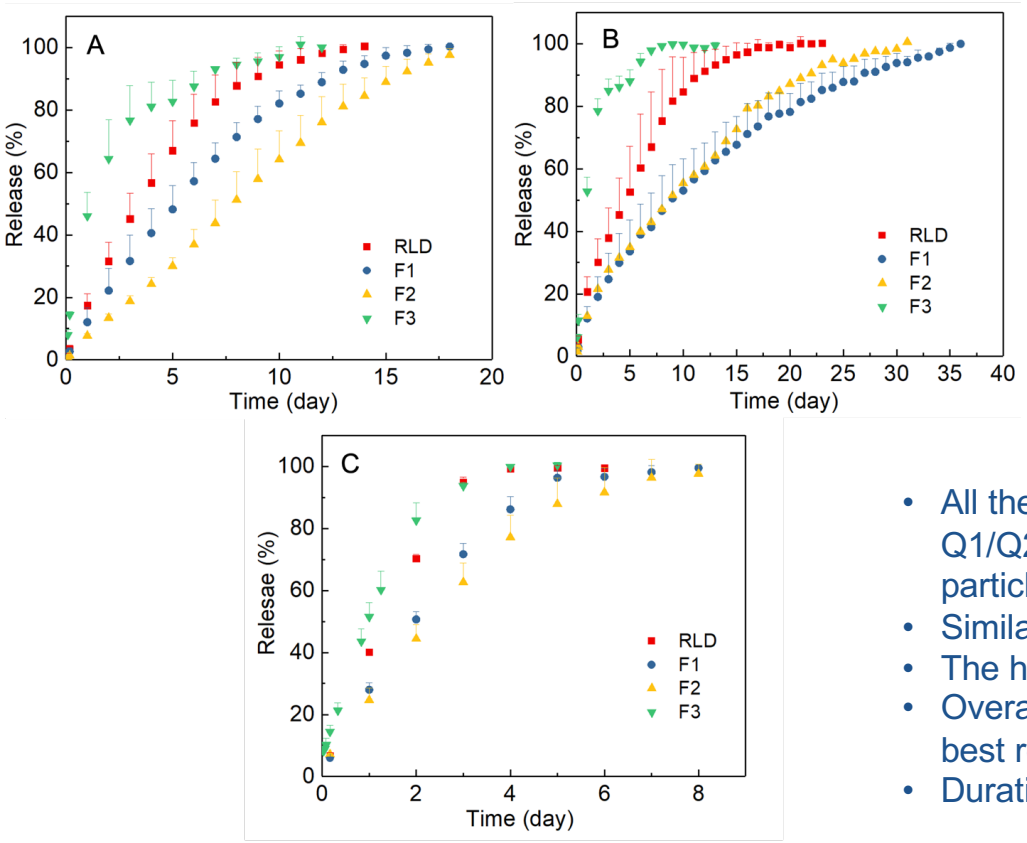
❑ Impact of flow rate on the release rate



- *In vitro* release profiles of the RLD obtained using USP apparatus 4 with semisolid adapters at different flow rates (2 mL/min, 4 mL/min and 8 mL/min). The release testing was performed in 500 mL of 1% w/v SDS in water at 37° C (mean ± SD, n=3).

- Flow rate had minimal impact on the drug release profiles of the LAI suspensions.
- In addition, the small error bars of the release profiles confirmed the excellent reproducibility of the method.

IVRT of the RLD and Prepared Q1/Q2 Equivalent LAI Suspensions Using Three Methods



- *In vitro* release profiles of the RLD and Q1/Q2 equivalent MPA suspensions (with different particle size) obtained using: **A)** USP apparatus 2 with enhancer cells; **B)** in-house designed devices; and **B)** USP apparatus 4 with semisolid adapters at 37° C (mean ± SD, n=3).

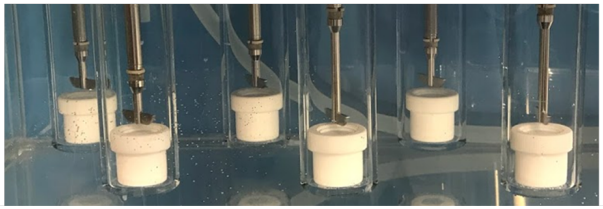
- All the methods had discriminatory capability to differentiate the Q1/Q2 equivalent MPA suspensions prepared with different particle sizes.
- Similar release trend was shown for all methods.
- The higher the particle size, the slower the drug release rate.
- Overall, the USP apparatus 4 with semisolid adapters showed the best reproducibility.
- Duration for all the formulations was extended as expected.

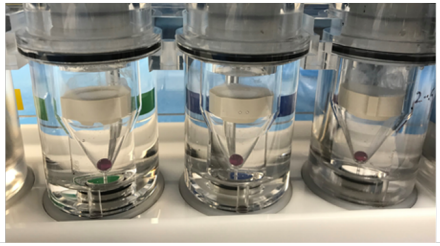
Q1/Q2 MPA Suspension Formulations for *In Vivo* Release Studies (Rabbit Model)

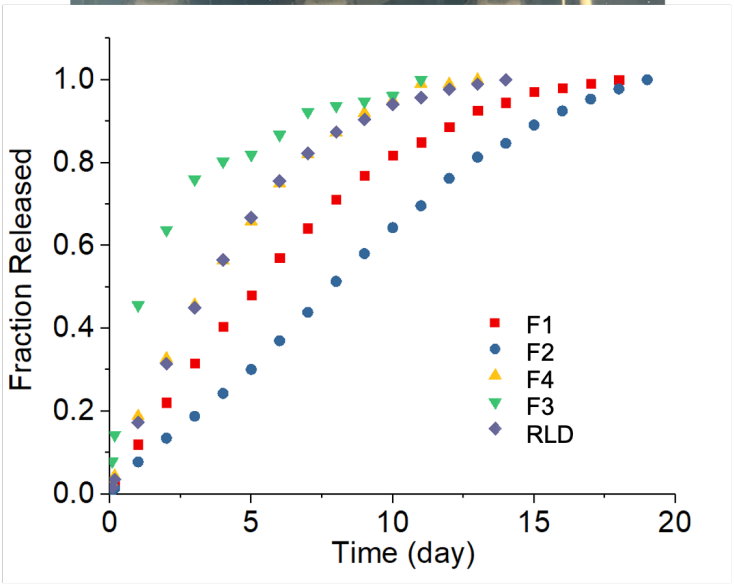
- **F1:** API was used as received
- **F2:** The API was recrystallized using acetone-water (1:1) system (water as anti-solvent). Following drying under vacuum at 40° C, the API was passed through a 45μm sieve. The API was added to the suspending media to achieve suspension F1.
- **F3:** processing based on F1 using probe sonication for 5 mins with 10% of pulse. The formulation underwent 10 s sonication, stop 1s.
- **F4:** Same as F1 except using different vendor of PEG3350 (Spectrum Chemical for F1 and BASF for F4)

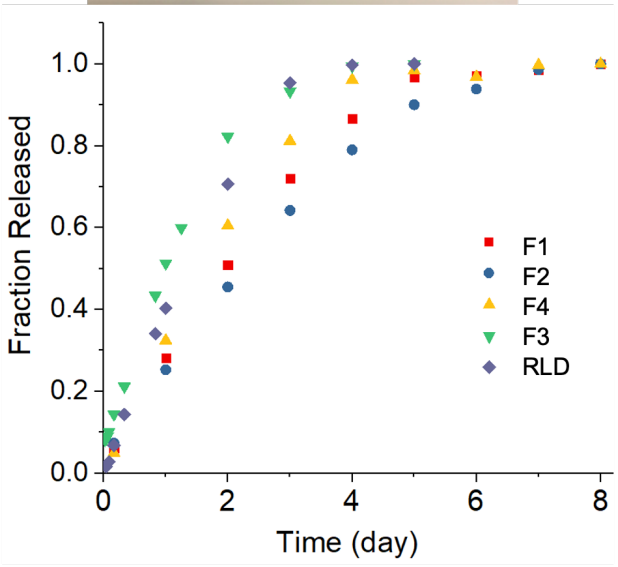
Formulation	Dv10	Dv50	Dv90	Span
F_1	7.21±0.42	**13.40±0.54**	24.09±0.74	1.26±0.04
F_2	8.73±0.31	**21.73±0.28**	41.08±0.53	1.49±0.04
F_3	0.69±0.33	**3.67±0.43**	10.13±0.99	2.61±0.44
F_4	7.00±0.13	**13.03±0.23**	23.44±0.37	1.26±0.01
RLD	10.37±0.99	**18.23±1.36**	30.61±1.78	1.11±0.05

Release Profiles of the Prepared LAI Suspensions and the RLD Using Different Methods

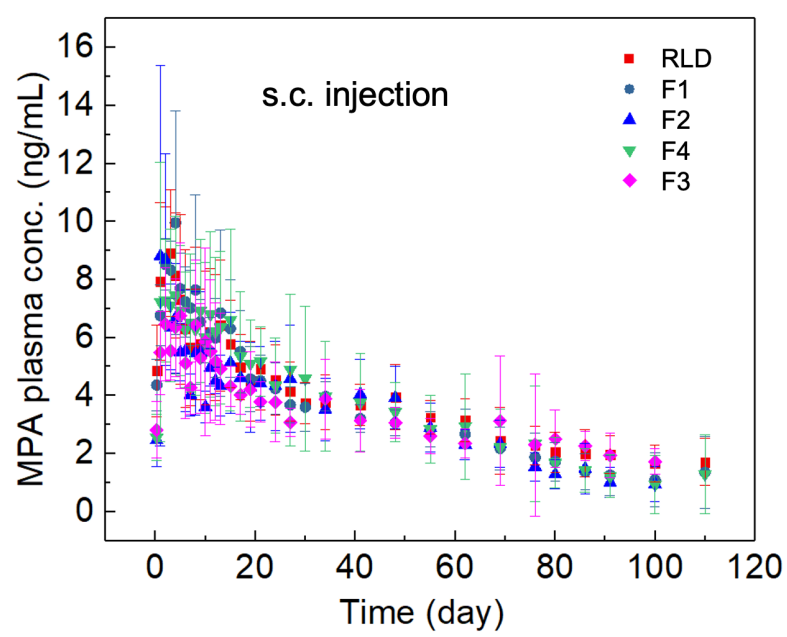


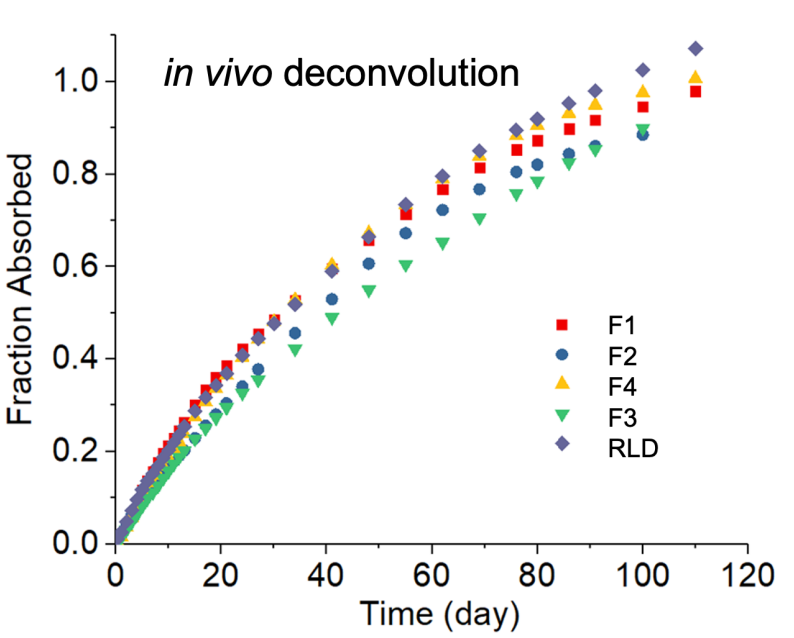






PK Profiles of LAI Suspensions

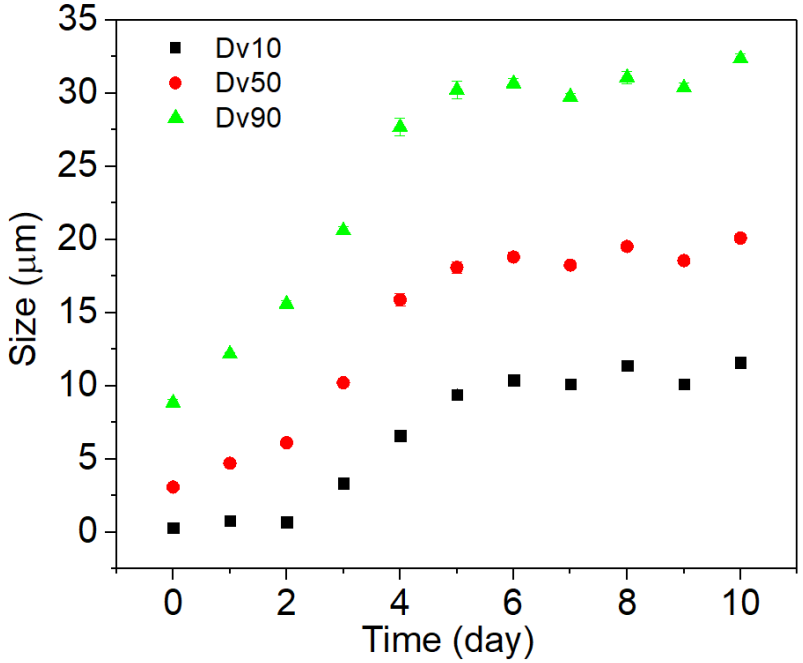


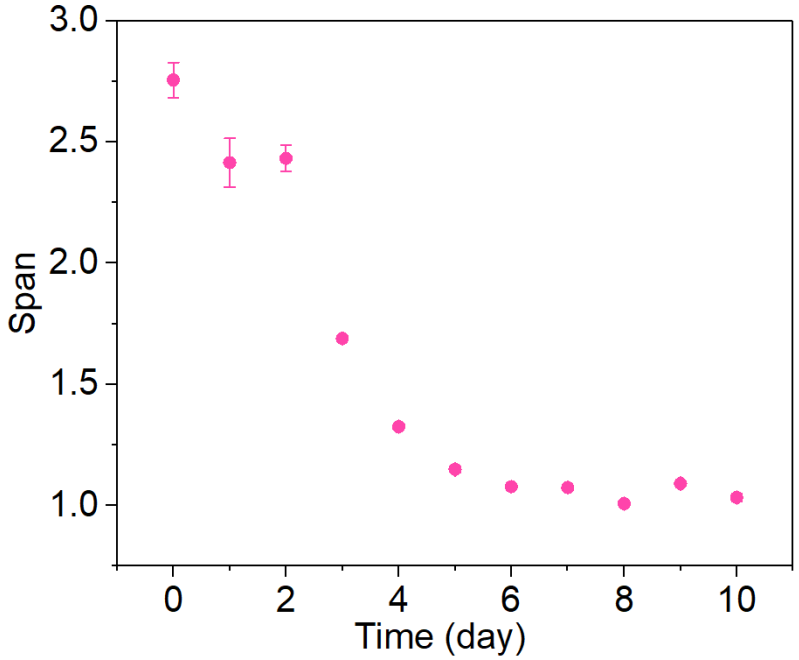


- The *in vivo* release of the prepared MPA suspensions and the RLD Depo-SubQ Provera 104® were investigated in female New Zealand White rabbits (n=6).
- F3 does not follow the rank order *in vivo.*

Particle Size of F3

- Particle size of F3 increased with time, stabilizing at around day 5 at ~20 μm. The Span data is consistent with the particle size change.
- This may be the reason why F3 does not follow the rank order *in vivo*.

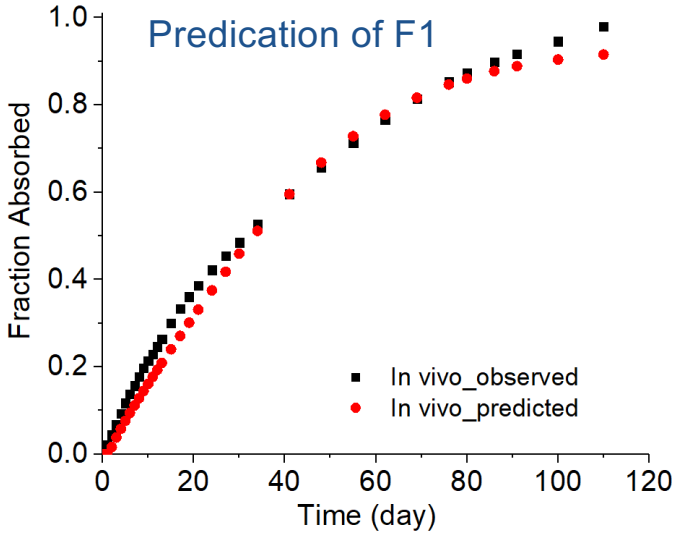




Development of IVIVCs for LAI Suspensions Using Conventional Method (WinNonlin®): USP App 2

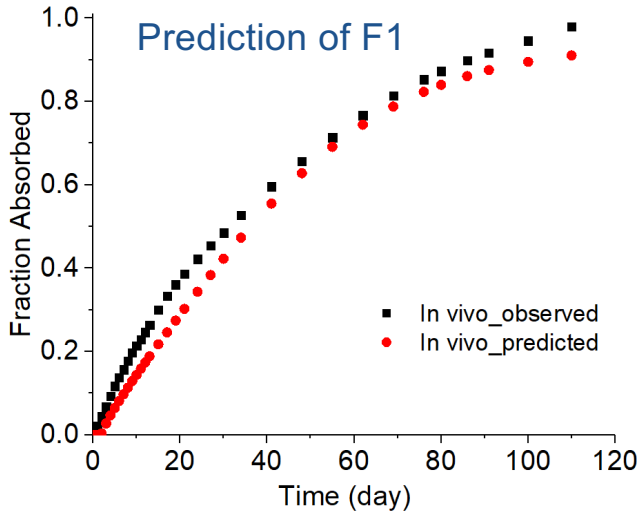
- Dissolution input: *in vitro* release data obtained using **USP apparatus 2 with enhancer cells.**
- Formulations: F1, F2 and RLD
- Dissolution Model: Double Weibull
- Reference formulation: F4

IVIVC Using F2 and RLD



Formulations	%PE in AUClast	%PE in Cmax
F1 (external)	-4.91	-14.65
F2 (internal)	1.93	-18.90
RLD (internal)	-13.38	26.26
Average (internal)	7.66	22.58

IVIVC Using F2, F4 and RLD



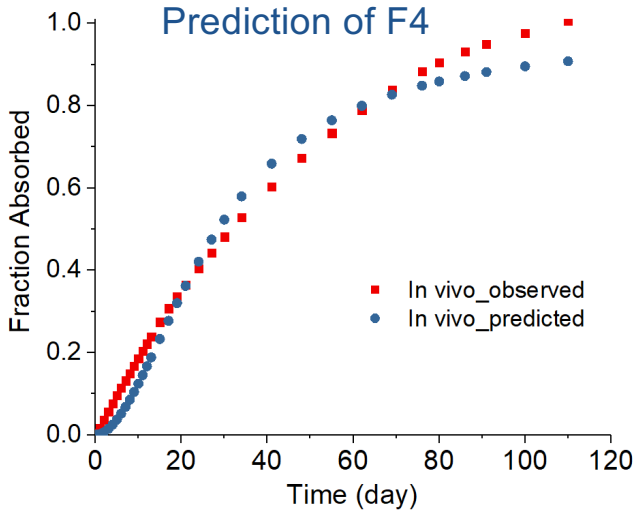
Formulations	%PE in AUClast	%PE in Cmax
F1 (external)	-4.20	-17.52
F2 (internal)	-4.35	-53.11
F4 (internal)	-5.59	69.38
RLD (internal)	-11.99	13.51
Average (internal)	7.31	45.34

Development of IVIVCs for LAI Suspensions Using Conventional Method (WinNonlin®): USP App 4

- Dissolution input: *in vitro* release data obtained using **USP apparatus 4 with semisolid adapters.**
- Formulations: F1, F2, F4 and RLD.
- Dissolution Model: Weibull (4 formulations)/Hill (3 formulations)
- Reference formulation: IV

IVIVC Using F1, and RLD

Dissolution Model: Hill

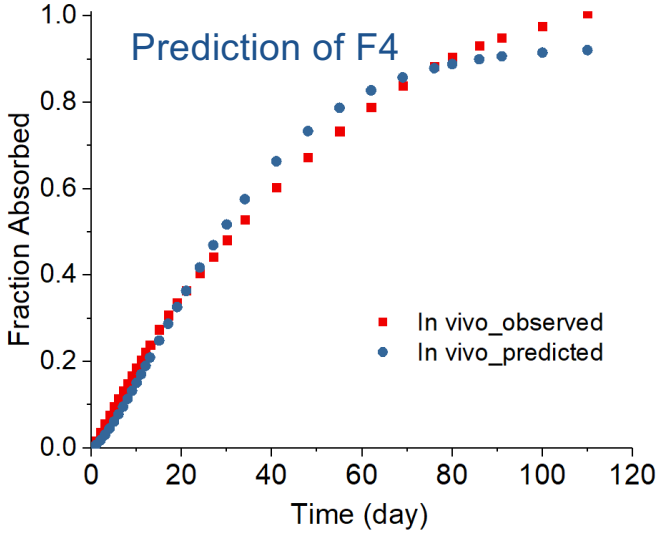


Formulations	%PE in AUClast	%PE in Cmax
F1 (internal)	-8.34	-36.58
F4 (external)	-9.81	7.66
RLD (internal)	-8.80	3.70
Average (internal)	8.57	20.14

IVIVC Using F1, F2, and RLD

IVIVC_F1, F2 and RLD

Dissolution Model: Weibull



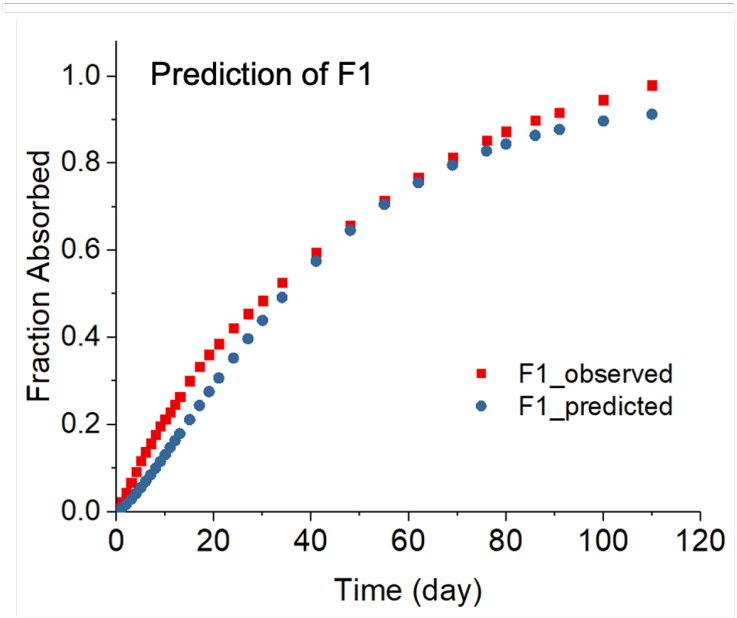
Formulations	%PE in AUClast	%PE in Cmax
F1 (internal)	-7.02	-41.40
F2 (internal)	-3.24	-42.35
F4 (external)	-9.07	-5.06
RLD (internal)	-11.17	-1.22
Average (internal)	7.15	28.32

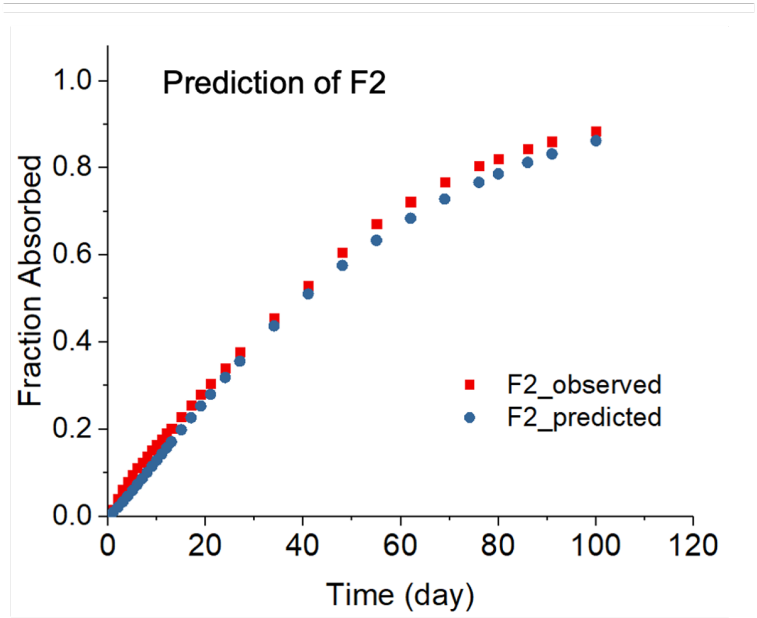
Representative Profiles of Predictions

IVIVC_F2, F4 and RLD

IVIVC_F1, F4 and RLD

Dissolution Model: Weibull





Summary

- Compendial methods with minor modifications used for *in vitro* release testing of LAI suspensions.
- Good discriminatory ability for Q1/Q2 equivalent LAI suspensions
- USP app 4 with semisolid adapters showed the best reproducibility, USP app 2 methods showed better discriminatory ability (larger margin).
- The developed *in vitro* release testing methods have long release durations, and may be more reflective of the *in vivo* release of LAIs.
- Level A IVIVCs were successfully developed using data obtained from USP app 4 with semisolid adapters.
- To increase our understanding of IVIVCs for LAI suspensions, other modeling such as PBPK should also be performed.

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