An in situ-forming, solid lipid/PLGA hybrid implant for long-acting antipsychotics

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1. Determination of risperidone solubility

A saturated solution of risperidone in saline was prepared at 37℃, and the sample was filtered with 0.45μm membrane before determination by HPLC.

Table S1. Solubility of risperidone in saline

solvent	Solubility (μ g/ml)
Saline	103.41±5.56

2. Diffusion test from dialysis membrane

The diffusion test through dialysis membrane was carried out as described in Materials and Methods section. As shown in Fig S1, the free risperidone diffused through the dialysis membrane in a very fast fashion, indicating that the membrane did not pose a diffusion barrier to the freely moving of risperidone.

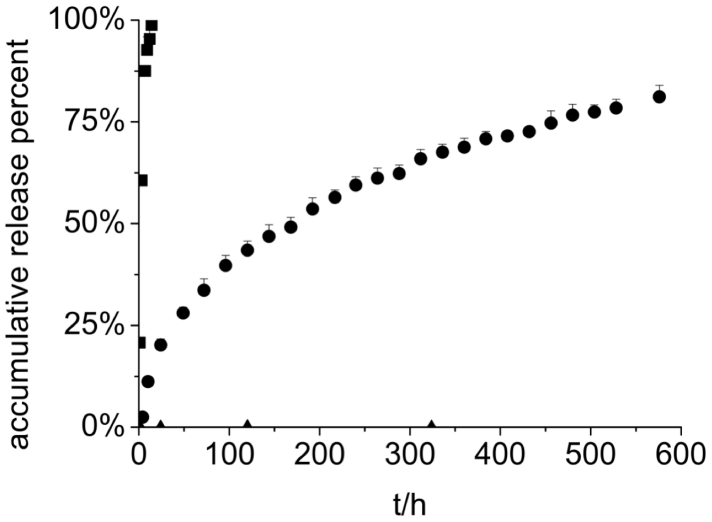

Fig S1. The release curve of risperidone. A. (●) P-I of risperidone; B. (■) risperidone solution; C. (▲) P-I without risperidone

3. Validation of HPLC method

3.1 Peak resolution test

The chromatographic condition was as described in the section of Materials and Methods. The risperidone was separated clearly from other compounds, and the peak resolution is > 1.5.

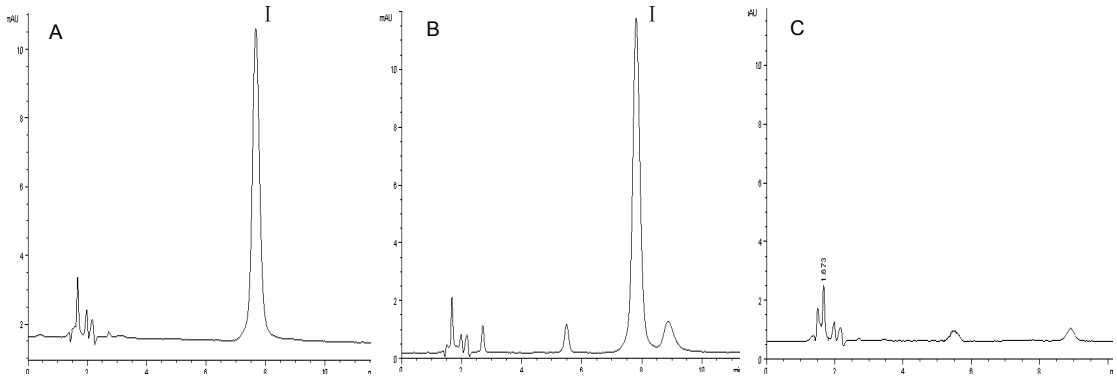

Fig S2. The HPLC chromatogram of risperidone (I). (A) risperidone saline solution. (B) in vitro release sample of preparation. (C) release medium sample of blank preparation.

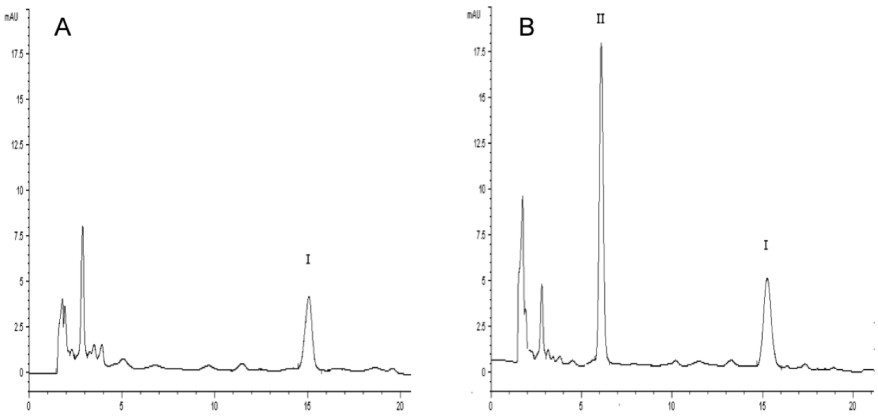

Fig S3. The HPLC chromatogram of plasma. (A) blank plasma containing internal standard; (B) rabbit plasma sample from PK study with addition of internal standard. (I, internal standard (clozapine); II, risperidon)

3.2 Preparation of the standard curve

Internal standard method was used for plasma analysis by HPLC, and the ratio of peak areas of and analyte (risperidone) and internal standard (clozapine), i.e., Ar/As. was used for quantitative analysis. A standard curve was obtained by plotting the peak area ratio (Ar/As) versus concentrations of samples (*c*), as shown below.

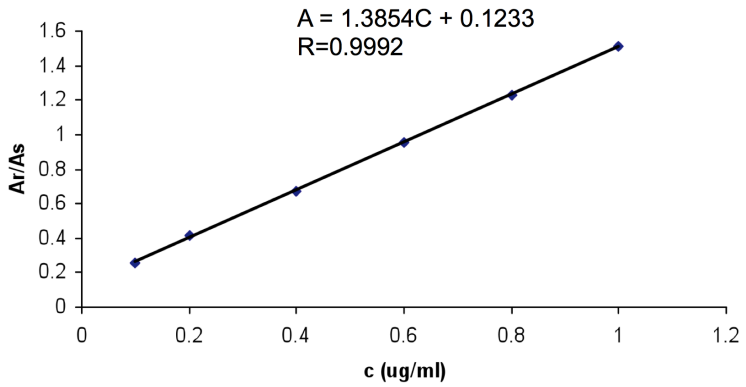

Fig S4. Standard curve

A = 1.3854C + 0.1233（r=0.9992）
Linear range: 0.1-1μg/ml

3.3 Precision study

Inter-day variability was tested by running the samples with varying concentrations (i.e., 0.2 μg/ml，0.6 μg/ml, and 1.0 μg/ml) at different time points within a day and calculating the relative standard deviation (RSD). Intra-day variability was determined by running samples at a daily base. The inter-day variability (RSD) of these samples was measured 2.35%，3.34%, and 2.37%，respectively, and the intra-day variability 4.19%，5.17%, and 3.92%, respectively.

3.4 Recovery

Table S2. The recovery of risperidone and clozapine in plasma

	Concentration (μg/ml)	Recovery (%)
risperidone	0.1	116.86±2.98
	0.2	109.86±2.35
	0.4	93.78±3.53
	0.6	90.79±3.34
	0.8	90.13±5.50
	1.0	89.08±2.37
clozapine	5.0	100.96±1.60

4. Muscle irritation scoring[1]

Table S3. Irritation scoring of intramuscular injection

Reaction Criteria	Score
No discernible gross reaction	0
Slight hyperemia and discoloration	1
Moderate hyperemia and discoloration	2
Distinct discoloration in comparison with the color of the surrounding area	3
Small areas of necrosis	4
Widespread necrosis, possibly involving the underlying muscle	5

Table S4. Category of irritation

Average Score	Grade
<0.4	None
0.5-1.4	Slight
1.5-2.4	Mild
2.5-3.4	Moderate
3.5-4.4	Marked
>4.5	Severe

[1] S.C. Gad. Irritation and local tissue tolerance studies in pharmaceutical safety assessment. In Preclinical development handbook: Toxicology, edited by S.C. Gad. John Wiley & Sons. Inc., Hoboken, New Jersey. 2008.