

***Macleaya cordata* helps improve the growth-promoting effect of chlortetracycline on broiler chickens**

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Materials and methods

Series of chlortetracycline (CTC) standard solutions at different concentrations (10, 50, 100, 500, 1000, 2000 ng/ml) were prepared by dissolving CTC hydrochloride with methyl alcohol. After diluted by high-performance liquid chromatography (HPLC) mobile phase, quantitation of CTC in standard solution was detected by HPLC and a standard linear curve was fitted. CTC concentrations in different tissues were measured by HPLC and quantified at 350 nm using a 5- μ m particle size Eclipse XDB-C18 column (4.6 mm \times 250 nm). The mobile phase was 0.01 M oxalic-acetonitrile-methyl alcohol solution (7:2:1) at a flow rate of 1 ml/min at 30 °C.

Table S1 Mobile phase of HPLC-MS/MS

Time (min)	A%	B%
	0.2% Methyl Alcohol	Acetonitrile
0	90	10
5	50	50
6	90	10
10	90	10

Table S2 Chromatographic parameters of HPLC-MS/MS

Flow velocity	300 µl/min
Chromatographic column	Waters Atlantis dC18 column (3.0 mm×150 mm, size: 3 µm)
Column temperature	35 °C
Wave length	350 nm
Injection volume	5 µl

Table S3 Mass spectrometry parameters of HPLC-MS/MS

Ion voltage	5500 V	
Auxiliary heater temperature	500 °C	
Curtain Gas	40 L/h	
Flow speed of desolventizzazione	Medium	
Ion-gas velocity	1. Atomization gas (gas1)	45 L/h
	2. Auxiliary gas (gas2)	25 L/h
Dwell time	20 ms	
Other parameters	ESI Source; Positive-ion scanning, etc.	

Table S4 Mass spectrometry parameters of qualitative and quantitative ion pair in HPLC-MS/MS

Parent ion, <i>m/z</i>	479.1
Daughter ion, <i>m/z</i>	444.1* 462.1
Declustering potential, eV	73 73
inlet voltage of collision cell, eV	4 4
Collision energy, eV	30 24
Export voltage of collision cell, eV	21 22

Note: *: quantitative ion

Results

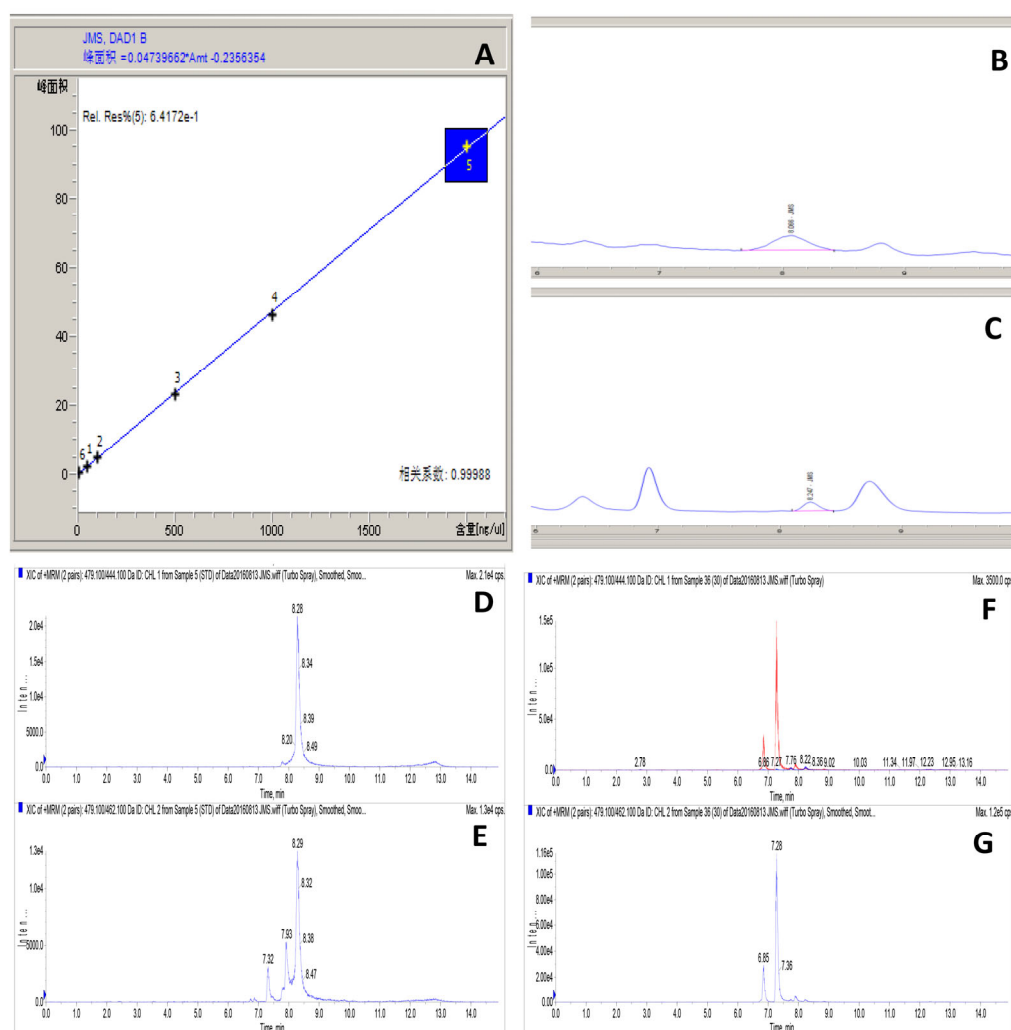


Fig. S1 Standard curve and peak figures

Note: curve standard (A) is only applied in HPLC. Areas of sample (B) and standard substance (C) in peak figures were converted to CTC concentrations using curve standard. Peak figures of blood samples (D, E) and standard substance (F, G) were display

Based on the relationship between peak areas and CTC concentration, a linear curve is obtained. Standard curve equation is $Y = 0.04739662X - 0.2356354$ ($R^2 = 0.99988$) with a detection limit at 2.08 ng/ml, and X and Y represent CTC concentration and peak area.