

# Pharmacokinetics and Tissue Distribution of Loratadine, Desloratadine and Their Active Metabolites in Rat based on a Newly Developed LC-MS/MS Analytical Method

## Authors

Yuxin Zhang<sup>1</sup>, Yihua Lu<sup>2</sup>, Lijuan Wang<sup>2</sup>, Yuan Tian<sup>2</sup>, Zunjian Zhang<sup>2</sup>

## Affiliations

- 1 Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, P. R. China
- 2 Key Laboratory of Drug Quality Control and Pharmacovigilance (Ministry of Education), China Pharmaceutical University, Nanjing, P. R. China

## Key words

drug metabolism, pharmacokinetics, absorption, loratadine, desloratadine, active metabolites, tissue distribution

received 14.05.2020

accepted 03.08.2020

published online 02.09.2020

## Bibliography

Drug Res 2020; 70: 528–540

DOI 10.1055/a-1233-5575

ISSN 2194-9379

© 2020. Thieme. All rights reserved.

Georg Thieme Verlag KG, Rüdigerstraße 14,  
70469 Stuttgart, Germany

## Correspondence

Prof. Dr. Zunjian Zhang

Key Laboratory of Drug Quality Control and Pharmacovigilance (Ministry of Education), China Pharmaceutical University

24 Tongjia Lane

Nanjing

210009

P. R. China

Tel.: +86-25-8327-1454,

zunjianzhangcpu@hotmail.com

## ABSTRACT

Loratadine (LOR) and its major metabolite, desloratadine (DL) are new-generation antihistamines. The hydroxylated metabolites of them, 6-OH-DL, 5-OH-DL and 3-OH-DL are also active because of their ability to inhibit binding of pyrilamine to brain H<sub>1</sub> receptors and a tendency for distributing to specific immune-regulatory tissues. In this study, a new validated LC-MS/MS method to simultaneously quantify LOR, DL, 6-OH-DL, 5-OH-DL and 3-OH-DL in plasma and tissues was established and applied to an investigation of their pharmacokinetics and target-tissue distribution tendency for the first time. Pharmacokinetics parameters in rat were measured and the results suggest that the body's exposure to active metabolites were much higher than to the prodrug with LOR, but much lower with DL. The tissue distribution study shows that LOR, DL and their active metabolites were widely distributed in the liver, spleen, thymus, heart, adrenal glands and pituitary gland. For immune-regulatory tissues, the concentrations of LOR, DL and their active metabolites in the spleen were much higher than in the thymus, which is related to the spleen, one of the sites where immune responses occur. LOR and its metabolites might inhibit immune-mediated allergic inflammation through the hypothalamic-pituitary-adrenal (HPA) axis. It was also found that the concentration of LOR in the heart was highest after liver and adrenal glands while those of DL, 6-OH-DL and 5-OH-DL in the liver, adrenal glands and spleen were all higher than those in the heart, which suggests that LOR may have a greater tendency to distribute in the heart than its metabolites.

## Introduction

Loratadine (LOR) is one of the second-generation antihistamines which are highly selective for H<sub>1</sub> receptors in periphery [1]. It can extensively metabolize to desloratadine (DL) by decarboethoxylation and subsequent oxidation and conjugation with glucuronic acid *in vivo* [2]. As the major active metabolite of LOR, DL has been developed as a third-generation antihistamine for its greater antihista-

minic potency, longer half-life and less first-pass effect compared to LOR [3]. Among the hydroxylated metabolites of LOR and DL, 6-hydroxy desloratadine (6-OH-DL), 5-hydroxy desloratadine (5-OH-DL) and 3-hydroxy desloratadine (3-OH-DL) are active because of their ability to inhibit binding of pyrilamine to rat brain H<sub>1</sub> receptors [4]. The structures of the above compounds are shown in ► Fig 1.

A number of analytical methods, including HPLC-UV [5], HPLC-FLD [6], HPLC-MS/MS [7, 8], turbulent flow chromatography (TFC)-MS/MS [9], and LC-MS/MS [10–15], have been established to simultaneously determine LOR and DL, DL and 3-OH-DL in biological fluids. Many of these methods require high sample volumes [5, 15] and so far there has been no reported study that simultaneously quantified LOR, DL, 6-OH-DL, 5-OH-DL and 3-OH-DL in biological samples.

However, the exposure to active metabolites and its proportional relationship with that to the prodrug *in vivo* remain unknown. It is important to investigate the role active metabolites play on the overall therapeutic effect and the possible reasons for the different therapeutic effects as well as adverse effects of LOR and DL. Therefore, this study developed a new highly selective and sensitive HPLC-MS/MS method to efficiently determine and quantify LOR, DL and their major active hydroxylated metabolites in rat plasma and tissues. The method was comprehensively validated in terms of selectivity, carryover, calibration curves, precision and accuracy, matrix effects, recovery, stability and dilution integrity, and was successfully applied to a pharmacokinetics and tissue-distribution investigation in rat after individual oral administration of LOR and DL.

## Materials and Methods

### Chemicals and reagents

Reference standards of LOR (100% purity), DL (99.7% purity) and diazepam (99.9% purity) were purchased from National Institutes for Food and Drug Control (Beijing, China). 6-OH-DL (99.7% purity), 5-OH-DL (99.8% purity) and 3-OH-DL (99.7% purity) were purchased from TLC Pharmaceutical Standards Ltd. (Vaughan, Canada). HPLC grade acetonitrile and methanol were from Merck (Darmstadt, Germany). Formic acid (HPLC grade) was from ROE SCIENTIFIC INC. (Newark, USA). Tetrahydrofuran (HPLC grade) was purchased from Aladdin (Shanghai, China). Analytical grade ammonium acetate and ethyl acetate were from Nanjing Chemical Reagents Co., LTD. (Nanjing, China). Double distilled water was purified by Milli-Q Water System of Merck Millipore (Darmstadt, Germany).

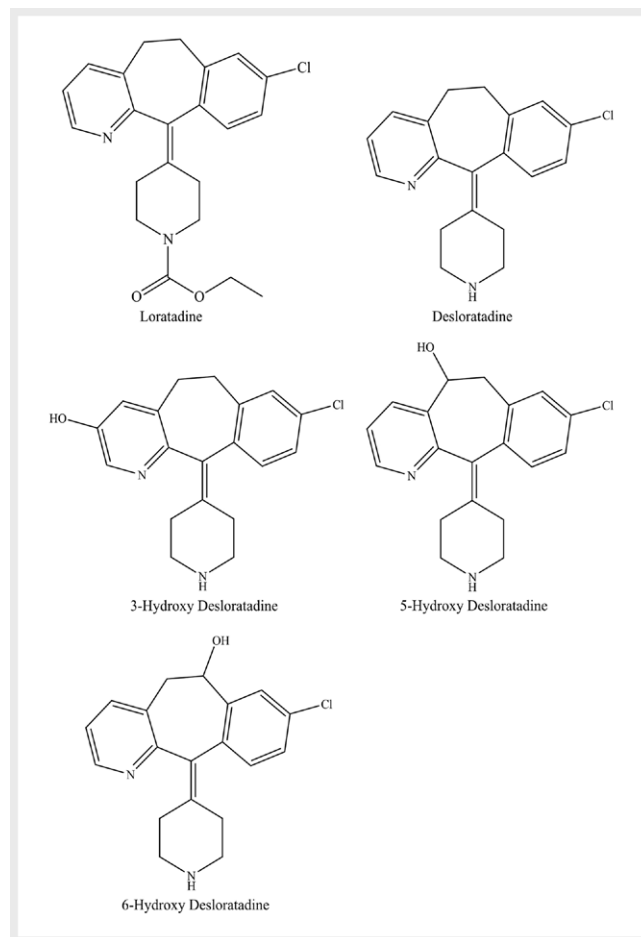
### LC-MS/MS instrument and conditions

Liquid chromatographic separation and mass spectrometric detection were done with Finnigan™ TSQ Quantum Discovery MAX™ LC-MS/MS system, comprising of Finnigan™ Surveyor auto-sampler, Finnigan™ Surveyor LC pump and triple-quadruple TSQ Quantum mass spectrometer. A ZORBAX RX-C<sub>8</sub> analytical column (2.1 × 150 mm, 5 μm) was used for chromatographic separation. Data were processed by Xcalibur 3.0 software. The mobile phase consisted of phase A (acetonitrile, containing 20% (V/V) tetrahydrofuran) and phase B (10 mM ammonium acetate, containing 0.2% (V/V) formic acid). A flow rate of 0.25 ml/min, 2 μl of injection volume and 30 °C of column temperature were chosen. The linear gradient elution program was set as follows: 0–4.5 min, 15% phase A; 4.51–10 min, 42% phase A; 10.01–20.00 min, 80% phase A; 20.01–30 min, 15% phase A. Analyses were performed in selected reaction monitoring (SRM) mode using electrospray ionization (ESI) in the positive ion mode, with parameter settings as follows: spray voltage 4800V, capillary temperature 300 °C, sheath gas pressure at 25 arbitrary units, auxiliary gas pressure at 5 arbitrary units, collision gas pressure at 1.0 mTorr, scan width 0.5 m/z and scan time 0.2 s.

### Preparation of standard solutions and quality control samples

Standard stock solutions (1 mg/ml) of LOR, DL, 6-OH-DL, 5-OH-DL, 3-OH-DL and diazepam (internal standard, IS) were prepared in methanol and stored at 4 °C. Standard working solutions were prepared by diluting serially from the standard stock solution.

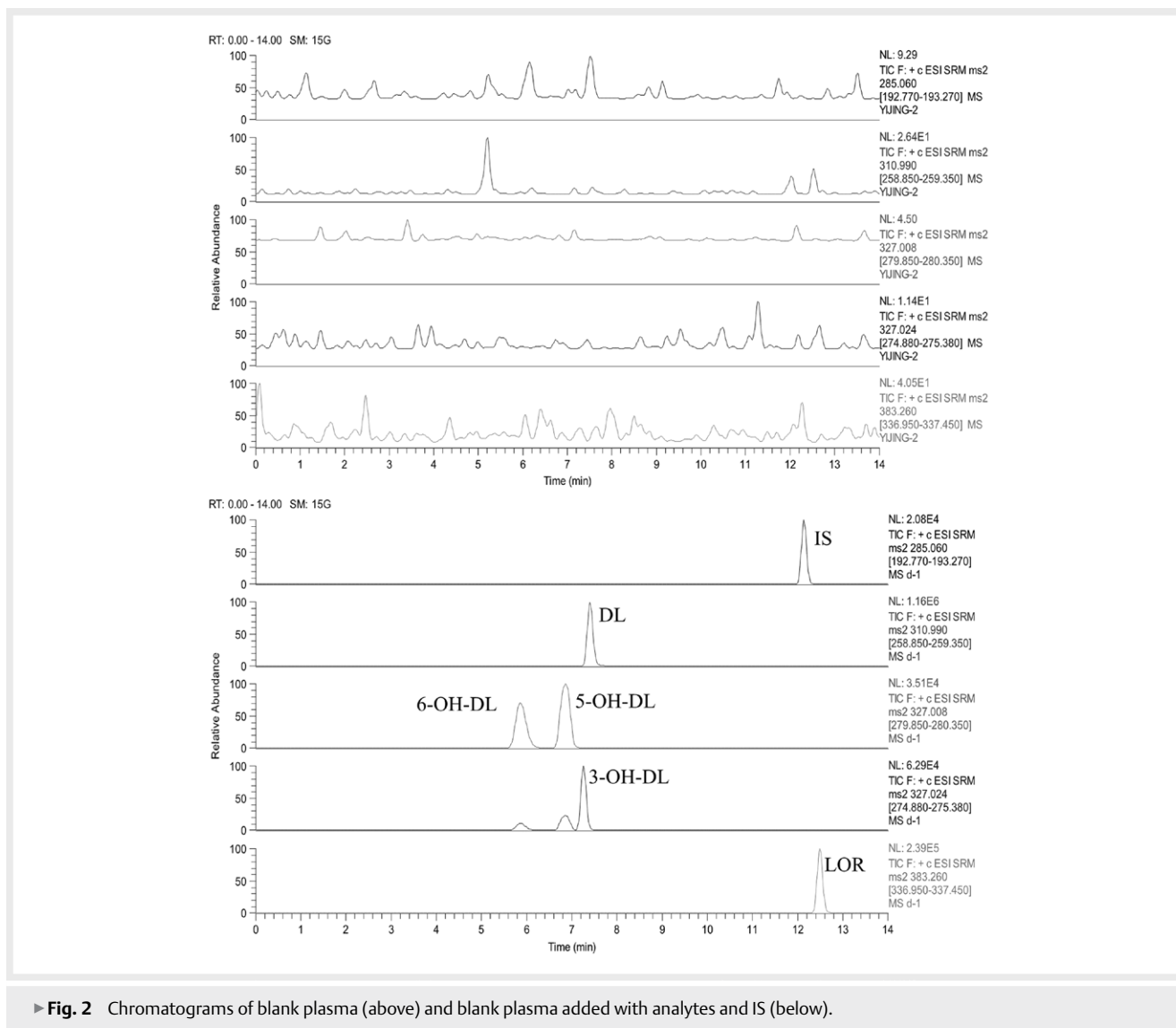
Calibration standards (CS) and quality control (QC) samples were prepared by adding appropriate amounts of standard working solution to blank plasma. The CS sample concentrations were 0.25, 0.5, 5, 12.5, 25, 50, 100, 200, 250 ng/ml for LOR; 0.5, 1, 10, 25, 50, 100, 200, 400, 500 ng/ml for DL; and 2, 5, 10, 20, 40, 80, 100 ng/ml for 6-OH-DL, 5-OH-DL and 3-OH-DL. The three QC sam-



► **Fig. 1** Structures of Loratadine, Desloratadine, 3-Hydroxy Desloratadine, 5-Hydroxy Desloratadine and 6-Hydroxy Desloratadine.

► **Table 1** Selected reaction monitoring transitions and MS parameters for analytes and IS.

Analytes	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
LOR	383.3	337.2	22
DL	311.0	259.1	20
3-OH-DL	327.0	275.1	22
5-OH-DL	327.0	280.1	21
6-OH-DL	327.0	280.1	21
IS	285.1	193.0	33



► **Fig. 2** Chromatograms of blank plasma (above) and blank plasma added with analytes and IS (below).

ple concentrations were 0.5, 50, 200 ng/ml for LOR, 1, 100, 400 ng/ml for DL, and 5, 20, 80 ng/ml for 6-OH-DL, 5-OH-DL and 3-OH-DL.

### Plasma and tissue sample preparation

An aliquot of 50  $\mu$ l of plasma sample, 5  $\mu$ l of IS working solution and 25  $\mu$ l of NaOH solution (0.1 mol/L) were put into a 1.5 ml Eppendorf tube and vortex-mixed for 1 min. Then the mixture was added with 300  $\mu$ l of ethyl acetate and thoroughly vortex-mixed for 1 min. After the sample was centrifuged at 16000 rpm (4°C) for 10 min, the supernatant was taken into another 1.5 ml Eppendorf tube and evaporated to dryness by nitrogen in a 37°C thermostatic water bath. The dry residue was redissolved in 150  $\mu$ l of the mobile phase and then centrifuged. The supernatant was injected into the LC system for analysis.

Each weighed liver, spleen, thymus and heart sample was homogenized in physiological saline solution (1:4, w/v), while the homogenate/saline ratio for adrenal glands and pituitary gland was 1:20 and 1:100 (w/v), respectively. The 5  $\mu$ l of IS working solution and 25  $\mu$ l of NaOH solution (0.1 mol/L) were added into each ho-

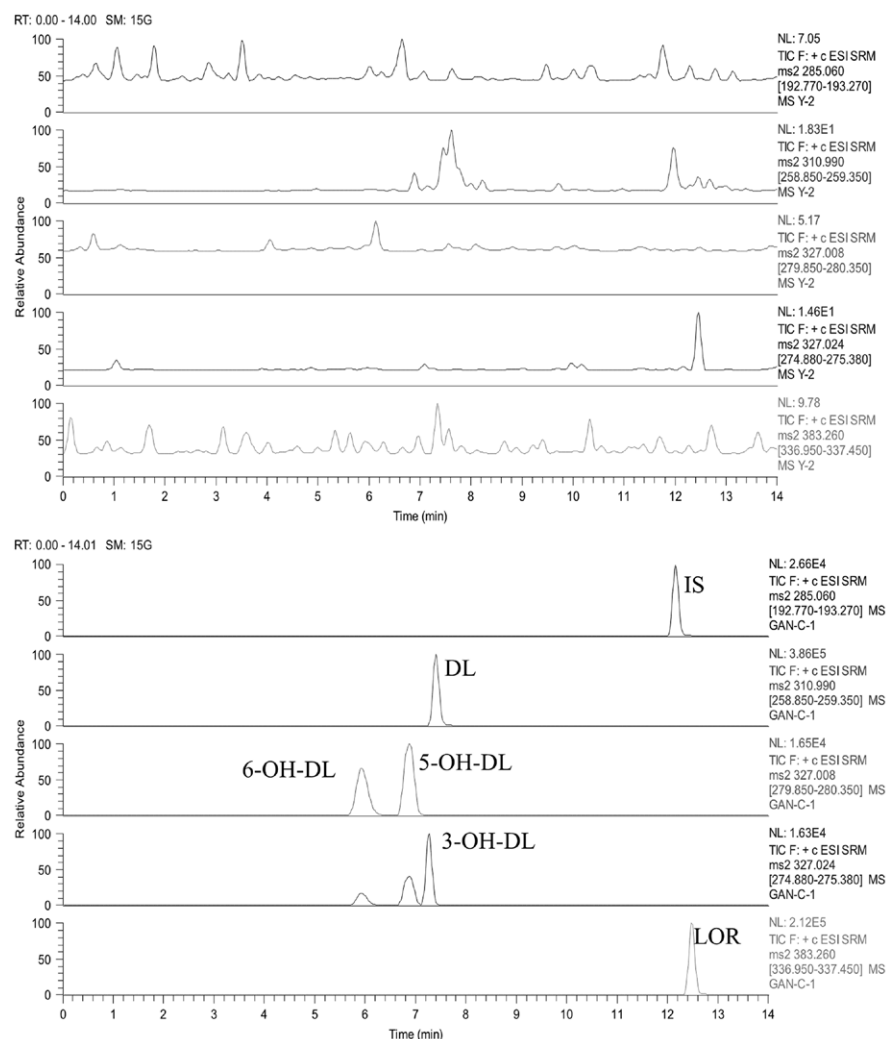
mogenate/saline solution. The heart homogenate sample (50  $\mu$ l) was pretreated by protein precipitation with methanol (300  $\mu$ l), while other tissue homogenate samples were pretreated in the same way as the plasma samples.

### Method validation

The method was validated in biological samples in terms of selectivity, carryover, calibration curves, precision and accuracy, matrix effects, recovery, stability and dilution integrity, mainly following the European Medicines Agency (EMA) Guideline on bioanalytical method validation [16].

Selectivity was assessed by screening six blank samples from individual rats. A response of interfering components at less than 20% of the low limit of quantification (LLOQ) for analytes and less than 5% for IS was considered acceptable.

Carryover was evaluated by injecting blank samples following high concentration samples. The carryover in blank samples should be lower than 20% of the LLOQ for analytes and lower than 5% for IS.



► **Fig. 3** Chromatograms of blank liver (above) and blank liver added with analytes and IS (below).

► **Table 2** Calibration curves and linearity ranges of analytes in plasma.

Analytes	Regression equation	Linearity range (ng/ml)	R <sup>2</sup>
LOR	$Y = 0.009153 + 0.07303 \times X$	0.25–250	0.9937
DL	$Y = 0.04431 + 0.2049 \times X$	0.5–500	0.9926
3-OH-DL	$Y = -0.007123 + 0.06696 \times X$	2–100	0.9911
5-OH-DL	$Y = -0.01350 + 0.04221 \times X$	2–100	0.9923
6-OH-DL	$Y = -0.03262 + 0.02990 \times X$	2–100	0.9910

Calibration curves were established in the concentration range of 0.25–250 ng/ml for LOR, 0.5–500 ng/ml for DL, 2–100 ng/ml for 6-OH-DL, 5-OH-DL and 3-OH-DL. The linearity was obtained by plotting the area ratios of analytes to IS (Y) versus the concentrations of analytes (X), weighted by  $1/Y^2$ . The deviations of back-calculated concentrations in the calibration curves should be within  $\pm 15\%$  except for LLOQ, which is within  $\pm 20\%$ .

Intra-day and inter-day precision and accuracy were evaluated by determining five replications at the LLOQ and three QC concentration levels in rat plasma, while intra-day precision and accuracy were evaluated at three QC concentration levels in rat tissues. The precision expressed as RSD and accuracy expressed as RE were accepted at within  $\pm 15\%$  except for LLOQ (within  $\pm 20\%$ ).

Matrix effects were investigated by analyzing six blank matrices from individuals using the analytical method. The matrix factor (MF) was obtained by calculating the ratio of peak area in the presence of matrix (determined by analyzing a blank matrix spiked with analytes after extraction) to peak area in the absence of matrix (determined by analyzing a solution of pure analytes). The matrix effect of IS was determined in the same way. The overall RSD should not be greater than 15%.

Absolute recoveries were calculated by comparing the peak area of analytes in extracted QC samples (at three QC concentration levels) with that of analytes in extracted blank matrix spiked with solutions at parallel concentrations. The recovery of IS was assessed by comparing the peak area of IS in extracted samples with that of blank matrix spiked with working solution of IS.

► **Table 3** Calibration curves and of analytes in tissues.

Tissues	Analytes	Regression equation	R <sup>2</sup>
Spleen	LOR	$Y = 0.01634 + 0.04255 \times X$	0.9950
	DL	$Y = 0.01834 + 0.04920 \times X$	0.9975
	3-OH-DL	$Y = -0.002804 + 0.003554 \times X$	0.9909
	5-OH-DL	$Y = -0.009851 + 0.01401 \times X$	0.9956
	6-OH-DL	$Y = -0.01300 + 0.009663 \times X$	0.9917
Thymus	LOR	$Y = 0.03722 + 0.03164 \times X$	0.9964
	DL	$Y = 0.4962 + 0.09122 \times X$	0.9959
	3-OH-DL	$Y = -0.008098 + 0.01932 \times X$	0.9972
	5-OH-DL	$Y = -0.02234 + 0.02241 \times X$	0.9929
	6-OH-DL	$Y = -0.02170 + 0.01476 \times X$	0.9936
Adrenal gland	LOR	$Y = 0.01018 + 0.05738 \times X$	0.9949
	DL	$Y = 0.01485 + 0.08232 \times X$	0.9963
	3-OH-DL	$Y = 0.002434 + 0.01041 \times X$	0.9912
	5-OH-DL	$Y = -0.01646 + 0.01715 \times X$	0.9974
	6-OH-DL	$Y = -0.01155 + 0.01052 \times X$	0.9957
Pituitary	LOR	$Y = 0.005822 + 0.05644 \times X$	0.9965
	DL	$Y = -0.01157 + 0.07339 \times X$	0.9955
	3-OH-DL	$Y = -0.009934 + 0.01553 \times X$	0.9920
	5-OH-DL	$Y = -0.01044 + 0.01495 \times X$	0.9987
	6-OH-DL	$Y = -0.01655 + 0.01127 \times X$	0.9916
Heart	LOR	$Y = 0.003769 + 0.02696 \times X$	0.9984
	DL	$Y = 0.007315 + 0.02323 \times X$	0.9949
	3-OH-DL	$Y = -0.004395 + 0.002865 \times X$	0.9920
	5-OH-DL	$Y = -0.009416 + 0.005881 \times X$	0.9925
	6-OH-DL	$Y = -0.009797 + 0.007851 \times X$	0.9934
Liver	LOR	$Y = 0.03280 + 0.03940 \times X$	0.9979
	DL	$Y = 0.05763 + 0.06815 \times X$	0.9936
	3-OH-DL	$Y = -0.001376 + 0.006333 \times X$	0.9906
	5-OH-DL	$Y = -0.01149 + 0.01608 \times X$	0.9946
	6-OH-DL	$Y = -0.01592 + 0.01328 \times X$	0.9922

Stability of stock solution and standard working solution, freeze and thaw stability, short-term and long-term stability, and on-automated stability were assessed to ensure that pretreatment and analysis conditions have no effect on the stability of analytes in solution and plasma samples. Stock solutions stored at 4 °C for 30 days and standard working solutions placed at room temperature for 10 h were investigated. The results of QC samples analyzed after replacement and storage in different conditions were compared to those of freshly-prepared QC samples.

Dilution integrity was evaluated by diluting samples whose concentrations were above the upper limit of quantification (ULOQ) with blank tissue homogenate. The accuracy and precision should be limited to  $\pm 15\%$ .

### Pharmacokinetics study

Twelve healthy male Sprague-Dawley rats (180–220 g) obtained from the Qinglongshan Experimental Animal Center (Nanjing, China) were randomly divided into two groups, each containing six rats. The LOR group and the DL group were administered a single oral dose of 50 mg/kg LOR (suspension in 0.5% aqueous sodium carboxymethyl-

cellulose) and 25 mg/kg DL (suspension in 0.5% aqueous sodium carboxymethylcellulose) by gavage, respectively. Blood samples (about 0.2 ml) were collected from the orbital venous plexus at 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36 h and 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 24, 36, 72 h for the LOR and DL group, respectively, and then placed in preheparinized tubes. The blood was centrifuged at 8000 rpm, 4 °C for 10 min and the supernatant separated into fresh an Eppendorf tube to store at -80 °C for further analysis.

### Tissue distribution study

Thirty SD rats were randomly divided into three groups: blank (n = 3), LOR group (n = 15) and DL group (n = 12). After a single oral administration of 50 mg/kg LOR and 25 mg/kg DL, respectively, rats from the LOR group were sacrificed at 0.25, 1, 3, 12 and 18 h and DL group at 1, 3, 12 and 18 h (3 rats each time). Spleen and thymus as target tissues, adrenal and pituitary glands as immune-regulatory tissues, heart as the toxicity tissue and liver as the metabolic tissue were collected and the blood rinsed with physiological saline and blotted on filter paper. All tissue samples were stored at -80 °C for further analysis.

## Result and Discussion

### LC-MS/MS condition optimization

MS analytical parameters were optimized by injecting analytes and IS standard solution into the ESI source directly through a TSQ Quantum syringe system using a compound optimization program. ► **Table 1** summarizes the selected reaction monitoring transitions and MS parameters of analytes and IS.

Since the same reaction monitoring ion was selected for 6-OH-DL and 5-OH-DL during analysis, it is important to separate the two. Comparing to ZORBAX SB-C<sub>3</sub>, ZORBAX Eclipse plus C<sub>8</sub>, Poroshell 120 PFP and Poroshell 120 Bonus RP, ZORBAX RX-C<sub>8</sub> column was found to have a tendency to separate 6-OH-DL and 5-OH-DL. The organic solvents, acids and buffers used in the mobile phase were optimized. It was found that an aqueous phase containing 10 mM ammonium acetate would obtain a good response. Investigating the resolution of 6-OH-DL and 5-OH-DL in different pHs, we found that both could be separated at pH 6.68 and pH 3.08 (0.2% (V/V) formic acid) and an addition of 0.2% (V/V) formic acid contributed to a sharper peak of isomers. Using acetonitrile as the organic phase, chromatographic peaks of analytes were sharper compared to methanol. On this basis, the resolution of 6-OH-DL and 5-OH-DL was above 1.5 when 20% (V/V) tetrahydrofuran (THF) was added to the acetonitrile.

### Sample pretreatment optimization

Rather than protein precipitation (PPT) and solid phase extraction (SPE), liquid-liquid extraction (LLE) was selected as the pretreatment method for its advantages of low cost, time saving and effective purification. Ethyl acetate, n-butyl alcohol and n-hexane were tested as extracting solvents. In the end, ethyl acetate was chosen for its highest extraction recovery. In order to reduce the interference of background contamination in the samples, different alkaline additives—0.1 mol/L NaOH, 0.1 mol/L Na<sub>2</sub>CO<sub>3</sub> and NH<sub>3</sub> · H<sub>2</sub>O—were investigated. The results showed that the recovery of analytes,

► **Table 4** The intra-batch and inter-batch accuracy and precision of analytes in plasma.

Analytes	Concentration (ng/ml)	Intra-batch (n=5)		Inter-batch (n=15)	
		Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
LOR	0.25	93.73	13.36	96.70	11.5
	0.5	102.6	6.54	98.72	8.21
	50	95.51	4.23	99.22	6.15
	200	96.72	3.41	100.4	6.11
DL	0.5	108.1	8.32	101.5	10.6
	1	106.6	4.68	99.07	9.23
	100	101.2	5.47	101.3	4.76
	400	107.4	4.73	105.8	5.26
3-OH-DL	2	99.24	6.55	102.1	7.88
	5	98.68	9.32	103.0	8.23
	20	101.0	9.28	102.4	6.61
	80	96.71	3.19	100.3	7.12
5-OH-DL	2	101.0	7.83	105.3	9.38
	5	94.24	7.85	95.33	8.15
	20	103.2	6.14	103.8	7.50
	80	93.22	4.89	92.28	4.58
6-OH-DL	2	99.75	8.41	99.38	9.11
	5	107.5	3.88	105.7	4.51
	20	104.8	5.70	100.0	7.59
	80	101.6	4.16	103.2	7.50

except 3-OH-DL, was above 90% when 0.1 mol/L NaOH was added, and lower than 90% with 0.1 mol/L Na<sub>2</sub>CO<sub>3</sub>, while the recovery of 3-OH-DL was lower than 50% with NH<sub>3</sub>·H<sub>2</sub>O. After further experimentation with different volumes (50, 25, and 10 μl) of 0.1 mol/L NaOH, the volume of 25 μl was chosen and the recovery was above 80% for 3-OH-DL and above 90% for the other analytes.

### Method validation

For the selectivity study in blank plasma and tissue samples, no interfering peaks from endogenous components showed at the retention time of analytes and IS. The representative chromatograms of blank plasma, blank plasma added with analytes and IS, blank liver and blank liver added with analytes and IS are shown in ► **Fig. 2** and ► **3**. The carryover of blank sample analyzed following high concentration samples was below 20% of the LLOQ and no greater than 5% of that of IS.

For the linearity study, the regression equation of calibration curves in plasma and tissues are listed in ► **Tables 2** and ► **3**. The results showed that the regression coefficients, R<sup>2</sup>, of all analytes in plasma and tissue homogenates were above 0.99 within the linearity range.

Intra-day and inter-day precision and accuracy of plasma QC samples at four concentration levels and tissue QC samples at three concentration levels are summarized in ► **Tables 4** and ► **5**. Accuracy and precision biases were all within ± 15% except for LLOQ, which is within ± 20%.

The recovery and matrix effects investigated at three QC concentrations are presented in ► **Tables 6–8**. The means of the matrix effects were within 96.78–107.8%, which suggest that the sam-

ple matrices had no significant effect on the ionization efficiency of analytes and IS. The mean recovery of LOR, DL, 5-OH-DL and 6-OH-DL was within 74.16–96.70% in plasma. The recovery of 3-OH-DL in plasma was 62.70–63.45% and stable at the three concentration levels. The mean recovery in the homogenates was as follows: 72.86–100.5% for LOR, 6-OH-DL and 5-OH-DL and 53.30–77.50% for DL and 3-OH-DL in spleen homogenates; 76.41–106.1% for the five analytes in thymus and heart; 84.16–94.14% for LOR, DL, 6-OH-DL and 5-OH-DL and 67.72–74.23% for 3-OH-DL in adrenal glands; 80.17–96.60% for the five analytes in pituitary gland; 74.66–108.9% for LOR, DL and 6-OH-DL and 50.23–76.64% for 5-OH-DL and 3-OH-DL in liver.

Stock solution, standard working solution and QC samples proved stable during pretreatment and analysis. ► **Table 9** shows the results of dilution integrity and suggests that diluted tissue QC samples could be reliably quantified.

### Application to pharmacokinetics

The developed method was implemented to determining plasma samples in pharmacokinetics studies. Trace 3-OH-DL could be detected in some plasma samples, but could not be accurately quantified because its concentration was lower than the LLOQ. Therefore, only LOR, DL, 6-OH-DL and 5-OH-DL were quantified in the plasma after individual oral administration of LOR and DL.

The plasma concentration-time profiles of LOR and its metabolites are presented in ► **Fig. 4a** and **b** and corresponding pharmacokinetic parameters listed in ► **Table 10**; for DL and its metabolites in ► **Fig. 5a** and **b** and corresponding pharmacokinetic parameters listed in ► **Table 11**. In rats administered with LOR orally,

► **Table 5** The intra-batch accuracy and precision of analytes in tissues.

Tissues	Analytes	Concentration (ng/ml)	Intra-batch (n=5)	
			Accuracy (%)	Precision (%)
Spleen	LOR	0.5	103.6	7.89
		50	92.07	5.87
		200	99.14	8.01
	DL	1	100.7	5.93
		100	99.51	9.03
		400	103.6	6.98
	3-OH-DL	5	103.8	2.71
		20	92.30	4.17
		80	93.39	8.63
	5-OH-DL	5	103.6	8.18
		20	92.07	6.42
		80	99.14	4.36
	6-OH-DL	5	100.7	4.10
		20	99.51	8.58
		80	103.6	10.72
Thymus	LOR	0.5	108.6	4.16
		50	107.5	4.81
		200	95.37	6.19
	DL	1	99.86	8.05
		100	104.5	4.65
		400	101.1	6.91
	3-OH-DL	5	97.04	6.89
		20	91.40	3.97
		80	96.26	10.45
	5-OH-DL	5	94.24	7.33
		20	105.0	8.32
		80	97.27	8.33
	6-OH-DL	5	91.19	5.35
		20	105.0	7.34
		80	100.3	5.98
Adrenal gland	LOR	0.5	98.80	11.81
		50	103.8	6.66
		200	104.0	2.26
	DL	1	104.3	9.19
		100	103.5	5.19
		400	97.40	2.43
	3-OH-DL	5	99.40	10.63
		20	104.0	8.70
		80	94.01	3.25
	5-OH-DL	5	92.60	6.78
		20	100.7	9.68
		80	98.69	4.11
	6-OH-DL	5	97.78	4.06
		20	107.7	3.40
		80	108.2	2.17

► **Table 5** Continued

Tissues	Analytes	Concentration (ng/ml)	Intra-batch (n=5)	
			Accuracy (%)	Precision (%)
Pituitary	LOR	0.5	102.4	9.12
		50	102.7	3.59
		200	106.9	4.60
	DL	1	100.3	6.05
		100	104.3	13.32
		400	100.7	12.34
	3-OH-DL	5	104.2	3.25
		20	104.7	9.56
		80	89.34	2.55
	5-OH-DL	5	100.8	5.98
		20	102.6	9.48
		80	99.43	1.70
	6-OH-DL	5	102.4	7.04
		20	102.7	7.92
		80	106.9	10.71
Heart	LOR	0.5	102.8	3.68
		50	97.53	9.05
		200	108.1	7.71
	DL	1	92.05	6.06
		100	102.2	3.70
		400	106.6	8.27
	3-OH-DL	5	97.47	7.87
		20	105.4	5.68
		80	104.3	7.64
	5-OH-DL	5	102.2	5.37
		20	104.5	6.38
		80	91.91	3.31
	6-OH-DL	5	97.99	5.14
		20	91.17	5.20
		80	103.1	4.40
Liver	LOR	0.5	100.6	6.89
		50	93.83	7.21
		200	91.67	3.23
	DL	1	98.68	12.41
		100	96.24	5.63
		400	87.72	3.17
	3-OH-DL	5	95.14	5.29
		20	93.45	10.28
		80	91.00	6.25
	5-OH-DL	5	97.24	5.11
		20	96.23	9.30
		80	102.9	7.60
	6-OH-DL	5	104.5	6.37
		20	102.0	10.81
		80	91.56	5.74

► **Table 6** The recovery of analytes and IS in plasma.

Analyte	Concentration (ng/ml)	recovery (n=5)	RSD (%)
LOR	0.5	87.75	6.68
	50	85.99	5.47
	200	88.16	1.54
DL	1	84.62	3.71
	100	84.52	1.55
	400	92.10	3.14
3-OH-DL	5	62.70	9.96
	20	63.30	3.61
	80	63.45	3.57
5-OH-DL	5	83.85	4.91
	20	83.02	2.11
	80	94.83	5.81
6-OH-DL	5	74.16	8.77
	20	79.11	1.91
	80	96.70	5.75
IS	50	95.95	6.07

AUC<sub>0-t</sub> (mean ± SD, µg/L · h) of LOR, DL, 6-OH-DL and 5-OH-DL were 446.72 ± 158.82, 3073.30 ± 537.81, 548.11 ± 174.35, 596.43 ± 128.94, respectively; the AUC<sub>0-t</sub> of DL, 6-OH-DL and 5-OH-DL were 687.97%, 122.70% and 133.51% of that of LOR, respectively, which suggests that the body's exposure to the active metabolites was higher than that to LOR after administration of the latter. In rats administered with DL orally, AUC<sub>0-t</sub> (mean ± SD, µg/L · h) of DL, 6-OH-DL and 5-OH-DL were 5880.17 ± 1499.36, 477.52 ± 104.48, 513.99 ± 121.22, respectively; the AUC<sub>0-t</sub> of 6-OH-DL and 5-OH-DL were 8.12% and 8.74% of that of DL, respectively, which suggests that the body's exposure to active metabolites were much lower than that of DL. Therefore, the active metabolites might have a greater effect on the overall efficacy in rats after oral administration of LOR comparing to that of DL.

As seen in ► **Fig. 4a** and **b**, DL, 6-OH-DL and 5-OH-DL had a double-peaks phenomenon in the LOR group. This phenomenon of DL was likely attributed to the mechanism that DL was produced in the liver and part of it was released into the blood by vein while another part was reabsorbed into the gastrointestinal tract by bile transfer or DL might exist in the enteric circulation. And the reason for the double-peaks phenomenon of 6-OH-DL and 5-OH-DL was speculated to be that the DL plasma concentration rose again *in vivo*. Due to individual differences, the SD values of pharmacokinetic parameters of LOR, DL, 6-OH-DL and 5-OH-DL were high. In the case of DL, this is possibly also because DL exhibits a pH-dependent efflux which could further induce variable absorption [17].

### Application to tissue distribution

The tissue homogenates were analyzed by a validated LC-MS/MS method. Trace 3-OH-DL could be detected in tissue homogenates except for thymus, but it could not be accurately quantified because its concentration was lower than the LLOQ. Therefore, only LOR, DL, 6-OH-DL and 5-OH-DL were quantified in tissue samples.

► **Table 7** The recovery of analytes in tissues.

Tissue	Analyte	Concentration (ng/ml)	recovery (n=5)	RSD (%)
Spleen	LOR	0.5	100.5	8.86
		50	91.34	12.19
		200	87.79	5.81
	DL	1	77.50	11.48
		100	67.37	6.70
		400	70.88	12.05
	6-OH-DL	5	81.30	4.65
		20	73.49	8.07
		80	78.33	5.30
	5-OH-DL	5	76.46	9.58
		20	82.77	7.91
		80	72.86	5.58
	3-OH-DL	5	56.96	13.70
		20	69.31	13.28
		80	81.16	101.4
Thymus	LOR	0.5	96.29	1.87
		50	97.81	4.52
		200	86.61	6.98
	DL	1	93.29	6.31
		100	95.11	11.27
		400	106.1	3.62
	6-OH-DL	5	81.61	7.40
		20	88.91	7.81
		80	105.8	5.46
	5-OH-DL	5	91.71	8.10
		20	86.78	6.94
		80	103.9	5.37
	3-OH-DL	5	81.04	3.44
		20	92.98	8.86
		80	90.85	5.20
Adrenal gland	LOR	0.5	85.96	3.52
		50	88.11	1.91
		200	89.43	1.38
	DL	1	92.15	4.81
		100	91.73	3.42
		400	90.35	5.13
	6-OH-DL	5	89.43	6.50
		20	94.14	5.58
		80	89.30	5.49
	5-OH-DL	5	84.16	9.64
		20	86.38	9.61
		80	88.28	4.58
	3-OH-DL	5	67.62	6.54
		20	71.92	5.73
		80	74.23	5.11



► **Table 7** Continued

Tissue	Analyte	Concentration (ng/ml)	recovery (n=5)	RSD (%)
Pituitary	LOR	0.5	95.38	0.86
		50	93.14	6.35
		200	86.63	3.54
		1	96.60	4.84
		100	89.90	6.54
		400	92.07	2.43
	6-OH-DL	5	90.29	4.63
		20	80.17	9.28
		80	87.73	1.62
	5-OH-DL	5	94.61	2.57
		20	80.17	5.81
		80	88.58	1.29
	3-OH-DL	5	90.76	3.08
		20	82.23	3.80
		80	80.34	2.75
Heart	LOR	0.5	95.80	10.09
		50	103.7	10.11
		200	89.56	2.06
	DL	1	82.40	2.49
		100	81.50	9.82
		400	92.06	8.04
	6-OH-DL	5	83.82	6.52
		20	92.64	8.77
		80	96.53	10.48
	5-OH-DL	5	83.07	12.10
		20	76.41	11.19
		80	81.13	9.67
	3-OH-DL	5	79.21	12.02
		20	79.76	8.25
		80	77.49	11.98
Liver	LOR	0.5	108.9	3.23
		50	97.89	3.24
		200	99.76	2.97
	DL	1	104.5	7.20
		100	91.01	8.75
		400	85.42	7.15
	6-OH-DL	5	74.66	6.26
		20	81.74	11.72
		80	76.36	14.11
	5-OH-DL	5	73.97	6.59
		20	76.64	9.13
		80	69.52	12.65
	3-OH-DL	5	50.23	12.50
		20	64.35	12.85
		80	66.32	13.71

► **Table 8** The matrix effect of analytes and IS in plasma.

Analyte	Concentration (ng/ml)	recovery (n=5)	RSD (%)
LOR	0.5	101.0	7.27
	50	100.9	9.91
	200	99.03	12.2
DL	1	104.2	5.60
	100	97.83	4.86
	400	105.0	5.57
3-OH-DL	5	101.0	7.21
	20	99.86	2.27
	80	96.78	7.55
5-OH-DL	5	97.98	4.03
	20	107.6	3.72
	80	98.47	6.24
6-OH-DL	5	98.53	6.95
	20	102.6	7.17
	80	102.9	4.73
IS	50	107.8	3.02

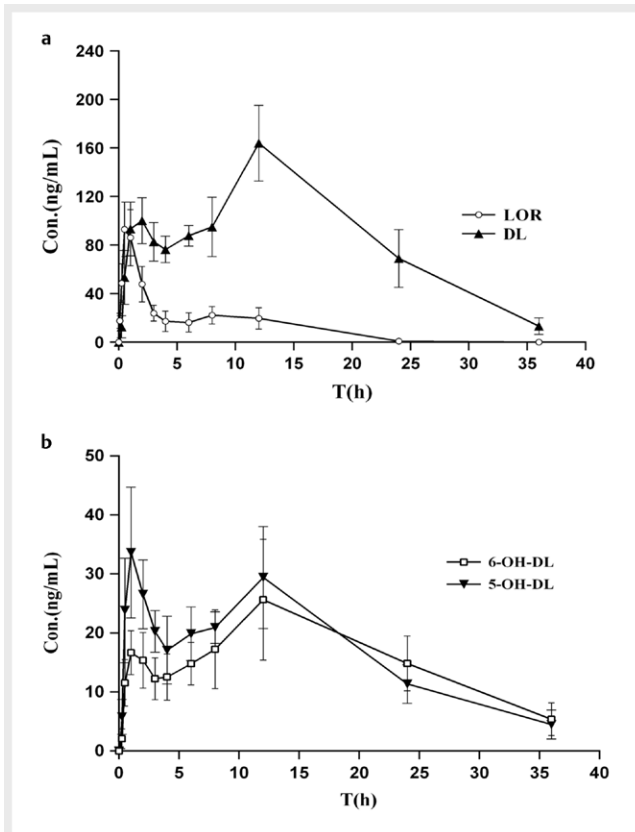
► **Table 9** The dilution integrity of analytes in tissues.

Tissues	Analytes	Concentration (ng/ml)	accuracy (n=5)	RSD (%)
Spleen	DL	5000	99.84	7.36
	5-OH-DL	150	104.9	8.43
Thymus	DL	4000	105.0	5.93
Adrenal gland	DL	2500	108.4	4.15
Pituitary	DL	500	87.74	1.82
Heart	DL	3000	93.30	3.61
Liver	LOR	2000	98.43	5.50
	DL	15000	98.76	9.59
	6-OH-DL	200	101.7	9.07
	5-OH-DL	1000	97.11	9.26

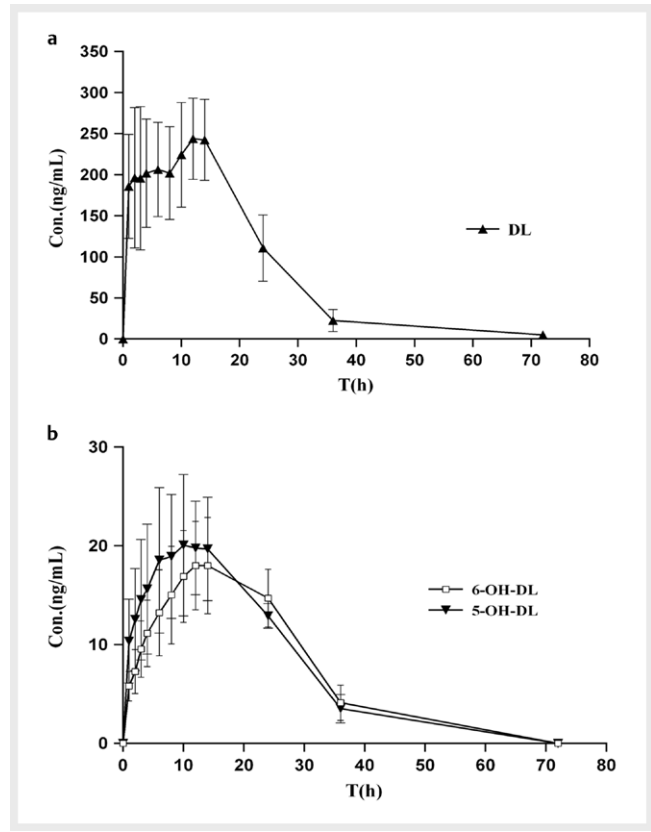
The concentration-time data of analytes in rat tissues are listed in

► **Tables 12–18.**

According to the results, LOR, DL, 6-OH-DL and 5-OH-DL were widely distributed in the liver, spleen, thymus, heart, adrenal glands and pituitary gland. For target tissues, their distribution in the spleen was higher than thymus, which was related to the fact that the spleen is an occurrence site of immune responses. The hypothalamic-pituitary-adrenal (HPA) axis can regulate the immune system by secreting glucocorticoids from the adrenal cortex, adrenocorticotropic hormone (ACTH) from the pituitary gland and corticotrophin-releasing hormone (CRH) from hypothalamus [18]. For immune-regulatory tissues, as LOR, DL and their active metabolites are distributed in the adrenal glands and pituitary gland, LOR and DL might inhibit immune-mediated allergic inflammation through the HPA axis. For the heart, neither LOR nor DL showed significant



► **Fig. 4** **a** Plasma concentration-time curves of LOR and its major metabolite DL after oral administration of 50 mg/kg of LOR in rats (n = 6) **b** Plasma concentration-time curves of LOR's metabolites 6-OH-DL and 5-OH-DL after oral administration of 50 mg/kg of LOR in rats (n = 6).



► **Fig. 5** **a** Plasma concentration-time curves of DL after oral administration of 25 mg/kg of DL in rats (n = 6) **b** Plasma concentration-time curves of DL's metabolites 6-OH-DL and 5-OH-DL after oral administration of 25 mg/kg of DL in rats (n = 6).

► **Table 10** Pharmacokinetic parameters of analytes (non atrioventricular model, n = 6) after oral administration of 50 mg/kg LOR in rats.

Parameters	LOR	DL	6-OH-DL	5-OH-DL
	Mean ± SD (%)	Mean ± SD (%)	Mean ± SD (%)	Mean ± SD (%)
AUC <sub>0-t</sub> (µg/L · h)	446.72 ± 158.82	3073.30 ± 537.81	548.11 ± 174.35	596.43 ± 128.94
AUC <sub>0-∞</sub> (µg/L · h)	514.94 ± 193.52	3239.22 ± 612.62	685.39 ± 165.75	697.88 ± 124.84
MRT <sub>0-t</sub> (h)	5.87 ± 1.44	13.85 ± 0.98	14.93 ± 2.18	12.79 ± 1.41
MRT <sub>0-∞</sub> (h)	8.92 ± 6.18	15.48 ± 2.03	23.56 ± 7.13	18.75 ± 5.73
t <sub>1/2</sub> (h)	6.24 ± 6.37	7.16 ± 2.46	13.83 ± 6.07	11.27 ± 5.07
T <sub>max</sub> (h)	0.67 ± 0.26	12.00 ± 0.00	8.33 ± 5.68	8.33 ± 5.68
V/F (L/kg)	723.96 ± 554.21	158.31 ± 37.59	1572.83 ± 858.37	1200.22 ± 649.43
CL/F (L/h/kg)	115.52 ± 62.52	16.06 ± 3.98	77.06 ± 20.95	73.5 ± 12.52
C <sub>max</sub> (µg/L)	96.99 ± 22.39	164.09 ± 31.27	26.98 ± 8.46	37.48 ± 9.15

► **Table 11** Pharmacokinetic parameters of analytes (non atrioventricular model, n=6) after oral administration of 25 mg/kg DL in rats.

Parameters	DL	6-OH-DL	5-OH-DL
	Mean ± SD(%)	Mean ± SD(%)	Mean ± SD(%)
AUC <sub>0-t</sub> (µg/L·h)	5880.17 ± 1499.36	477.52 ± 104.48	513.99 ± 121.22
AUC <sub>0-∞</sub> (µg/L·h)	5951.12 ± 1515.79	562.41 ± 123.79	579.75 ± 112.26
MRT <sub>0-t</sub> (h)	15.36 ± 2.61	17.52 ± 2.41	16.10 ± 2.11
MRT <sub>0-∞</sub> (h)	16.02 ± 2.83	24.06 ± 7.93	21.33 ± 6.52
t <sub>1/2</sub> (h)	8.49 ± 2.48	13.43 ± 6.19	12.18 ± 5.74
T <sub>max</sub> (h)	10.83 ± 4.12	12.67 ± 1.63	10.33 ± 2.94
V/F (L/kg)	52.17 ± 13.75	855.69 ± 281.79	758.27 ± 305.66
CL/F (L/h/kg)	4.38 ± 0.88	46.75 ± 12.69	44.46 ± 8.38
C <sub>max</sub> (µg/L)	254.31 ± 45.72	19.01 ± 4.63	21.59 ± 6.13

► **Table 12** Concentration-time data of LOR in tissues after oral administration of LOR in rats (Mean(ng/ml) ± SD(%), n=3).

Tissues	0.25h	1h	3h	12h	18h
Spleen	151.93 ± 146.77	98.89 ± 44.61	46.05 ± 41.68	4.12 ± 4.07	0.35 ± 0.61
Thymus	23.07 ± 15.67	83.38 ± 25.39	26.90 ± 19.30	0.87 ± 1.51	4.51 ± 7.81
Adrenal gland	1155.05 ± 636.89	1482.37 ± 1378.36	3361.55 ± 2728.76	56.40 ± 42.79	73.10 ± 46.12
Pituitary	91.89 ± 56.66	113.05 ± 48.05	74.76 ± 20.46	9.29 ± 16.09	n. d.
Heart	161.30 ± 95.11	217.79 ± 30.96	99.29 ± 46.74	21.58 ± 6.63	9.13 ± 5.59
Liver	3179.15 ± 2672.31	994.88 ± 655.00	264.65 ± 197.80	12.03 ± 11.22	6.56 ± 3.03

► **Table 13** Concentration-time data of DL in tissues after oral administration of LOR in rats (Mean (ng/ml) ± SD(%), n=3).

Tissues	0.25h	1h	3h	12h	18h
Spleen	265.79 ± 103.92	3774.02 ± 287.48	10356.27 ± 2567.74	14373.70 ± 6085.51	11110.98 ± 3635.04
Thymus	13.00 ± 12.18	578.21 ± 115.76	1824.63 ± 206.12	4958.59 ± 670.31	3899.45 ± 1959.73
Adrenal gland	387.21 ± 179.74	6309.71 ± 828.89	16596.79 ± 1368.44	23858.25 ± 4166.16	13771.79 ± 10866.08
Pituitary	177.17 ± 64.51	2894.36 ± 586.22	10550.07 ± 929.75	18322.74 ± 3835.90	13956.13 ± 7092.19
Heart	321.84 ± 209.27	3220.77 ± 1226.74	5585.49 ± 512.24	6958.95 ± 2401.89	2866.97 ± 982.39
Liver	10910.13 ± 5981.45	18025.00 ± 5783.07	21458.20 ± 307.32	24700.93 ± 6569.97	10164.93 ± 787.89

► **Table 14** Concentration-time data of 6-OH-DL in tissues after oral administration of LOR in rats (Mean(ng/ml) ± SD(%), n=3).

Tissues	0.25h	1h	3h	12h	18h
Spleen	3.10 ± 5.38	137.12 ± 18.02	165.27 ± 43.01	191.31 ± 45.50	123.85 ± 16.17
Thymus	n. d.	32.88 ± 3.37	82.02 ± 23.22	109.98 ± 28.98	69.23 ± 15.91
Adrenal gland	n. d.	211.08 ± 25.63	332.02 ± 107.78	399.98 ± 95.03	251.98 ± 71.03
Pituitary	n. d.	72.87 ± 126.22	422.17 ± 161.49	623.61 ± 94.42	453.43 ± 73.82
Heart	7.14 ± 6.49	125.34 ± 20.05	143.86 ± 48.78	150.18 ± 26.30	93.69 ± 15.40
Liver	91.22 ± 63.77	329.61 ± 317.45	253.70 ± 77.01	378.14 ± 302.52	270.51 ± 79.53

► **Table 15** Concentration-time data of 5-OH-DL in tissues after oral administration of LOR in rats (Mean(ng/ml) ± SD(%), n=3).

Tissues	0.25h	1h	3h	12h	18h
Spleen	23.31 ± 7.98	336.00 ± 59.57	410.39 ± 187.62	413.33 ± 118.65	217.44 ± 29.63
Thymus	n. d.	87.58 ± 24.99	165.31 ± 55.79	188.78 ± 65.76	107.21 ± 25.38
Adrenal gland	30.66 ± 26.61	595.09 ± 110.53	843.67 ± 226.54	766.16 ± 235.29	406.67 ± 112.22
Pituitary	n. d.	407.77 ± 103.89	915.73 ± 408.44	1050.65 ± 286.40	626.09 ± 183.08
Heart	31.02 ± 12.07	325.57 ± 88.17	304.25 ± 100.30	216.89 ± 80.24	104.64 ± 25.49
Liver	601.16 ± 764.94	1385.21 ± 974.45	1081.69 ± 668.51	289.39 ± 104.88	241.87 ± 70.91

► **Table 16** Concentration-time data of DL in tissues after oral administration of DL in rats (Mean(ng/ml) ± SD(%), n = 3).

Tissues	1h	3h	12h	18h
Spleen	10 663.39 ± 970.39	24 304.50 ± 8983.61	31 232.58 ± 5291.76	8792.61 ± 2411.54
Thymus	1226.95 ± 396.14	6313.42 ± 1941.60	12 556.65 ± 1001.22	8168.19 ± 3930.37
Adrenal gland	19971.88 ± 2358.14	38 575.00 ± 13947.57	43 612.87 ± 4550.03	24 298.57 ± 14 491.86
Pituitary	8436.26 ± 2078.85	22 352.97 ± 7081.29	47 754.03 ± 3588.28	28 402.12 ± 9362.43
Heart	5312.79 ± 1431.59	9595.89 ± 1777.75	9933.44 ± 1385.21	7585.56 ± 3502.77
Liver	39 160.47 ± 13225.75	45 963.38 ± 11760.76	48 003.14 ± 9181.43	20 347.85 ± 11903.83

► **Table 17** Concentration-time data of 6-OH-DL in tissues after oral administration of DL in rats (Mean(ng/ml) ± SD(%), n = 3).

Tissues	1h	3h	12h	18h
Spleen	43.46 ± 9.47	82.67 ± 28.92	155.10 ± 49.78	115.25 ± 16.32
Thymus	10.07 ± 1.00	32.40 ± 9.93	82.90 ± 35.54	62.46 ± 13.01
Adrenal gland	106.73 ± 17.34	204.46 ± 31.67	273.40 ± 76.43	341.35 ± 47.53
Pituitary	n. d.	265.68 ± 12.82	575.24 ± 233.33	619.44 ± 76.48
Heart	30.08 ± 6.62	73.32 ± 20.70	103.53 ± 32.15	86.75 ± 7.98
Liver	87.31 ± 54.05	226.85 ± 92.62	454.12 ± 247.73	330.49 ± 46.26

► **Table 18** Concentration-time data of 5-OH-DL in tissues after oral administration of DL in rats (Mean ± SD(%), n = 3).

Tissues	1h	3h	12h	18h
Spleen	87.65 ± 27.93	213.04 ± 98.77	335.06 ± 83.81	180.38 ± 37.45
Thymus	12.93 ± 0.70	44.81 ± 20.64	152.31 ± 69.99	86.89 ± 17.57
Adrenal gland	173.79 ± 10.90	401.08 ± 129.43	552.22 ± 197.24	528.04 ± 108.34
Pituitary	n. d.	298.22 ± 127.81	1169.40 ± 623.61	650.49 ± 30.42
Heart	48.07 ± 8.32	135.91 ± 53.23	180.52 ± 76.44	105.23 ± 13.32
Liver	149.29 ± 95.99	423.57 ± 301.16	808.15 ± 597.15	297.71 ± 75.64

cardiotoxicity at clinical doses according to relevant studies [18, 19], but there were 58 cases of adverse events from LOR and 35 cases from DL, excluding concomitant drug interactions, reported across Europe in the years of 2004–2011 [19–21]. In the tissue distribution of LOR, we found that the concentration of LOR in the heart was the highest except for liver and adrenal glands, while the concentrations of DL, 6-OH-DL and 5-OH-DL in the liver, adrenal glands and spleen were all higher than that in the heart, which suggests that LOR may have a greater tendency to distribute in the heart than its metabolites.

## Conclusions

A LC-MS/MS analysis for the simultaneous determination of LOR, DL, 6-OH-DL, 5-OH-DL and 3-OH-DL in rat plasma and tissues was developed and validated for the first time and would provide a better analytical alternative for clinical drug monitoring and bioequivalence studies of LOR and DL. The established method was successfully applied to a pharmacokinetics and tissue distribution investigation of LOR, DL and their active metabolites in rats. This study showed for the first time the pharmacokinetic parameters of 6-OH-DL and 5-OH-DL *in vivo* and the proportional relationship of exposure between the active metabolites and the prodrug. According to the results, LOR and DL could rapidly metabolize *in vivo* and the active metabolites might have a greater effect on the overall effi-

cacy of LOR and less effect on the overall efficacy of DL. Therapeutic effects of both LOR and DL were likely related to the spleen which is the occurrence site of immune responses and the inhibition of immune-mediated allergic inflammation through the HPA axis, and the risk of cardiac toxicity of LOR was bigger than that of active metabolites because of its greater tendency to distribute in the heart. Since the possible reasons of different therapeutic and adverse effects of LOR and DL are still unclear, these conclusions may provide a theoretical support for the clinical safety and further exploration of the toxicity mechanism of LOR and DL as well as possible development of 6-OH-DL and 5-OH-DL as new drugs.

## Sources of Funding

This work was financially supported by the National Major Science and Technology Projects of China(2017ZX09101001) and the Double First-Class University Project (CPU2018GY33).

## Acknowledgments

The authors would like to thank all subjects for their participation.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- [1] Appeals U C O. Second-generation antihistamines have similar efficacy in allergic rhinitis, but their tolerability and metabolism differ. *Drugs & Therapy Perspectives* 2007; 23 (10): 17–19
- [2] Ramanathan R, Alvarez N, Su AD et al. Metabolism and excretion of loratadine in male and female mice, rats and monkeys. *Xenobiotica* 2005; 35 (2): 155–189
- [3] Ramanathan R, Reyderman L, Kulmatycki K et al. Disposition of loratadine in healthy volunteers. *Xenobiotica* 2007; 37 (7): 753
- [4] FDA. Drug Approval Package [EB/OL]. (2001-11-20) [2018-06-10]. [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2001/21-165\\_Clarinex.cfm](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/21-165_Clarinex.cfm)
- [5] Yin OQ, Shi X, Chow MS. Reliable and specific high-performance liquid chromatographic method for simultaneous determination of loratadine and its metabolite in human plasma. *Journal of Chromatography B* 2003; 796 (1): 165–172
- [6] Belal F, El-Razeq SA, El-Awady M et al. Rapid micellar HPLC analysis of loratadine and its major metabolite desloratadine in nano-concentration range using monolithic column and fluorometric detection: Application to pharmaceuticals and biological fluids. *Chemistry Central Journal* 2016; 10 (1): 79
- [7] Vlase L, Imre S, Muntean D et al. Determination of loratadine and its active metabolite in human plasma by high-performance liquid chromatography with mass spectrometry detection. *Journal of Pharmaceutical & Biomedical Analysis* 2007; 44 (3): 652–657
- [8] Srinubabu G, Patel RS, Shedbalkar VP et al. Development and validation of high-throughput liquid chromatography-tandem mass spectrometric method for simultaneous quantification of loratadine and desloratadine in human plasma. *Journal of Chromatography B* 2007; 860 (2): 202–208
- [9] Dridi D, Attia MB, Sani M et al. Circadian Time-Effect of Orally Administered Loratadine on Plasma Pharmacokinetics in Mice. *Chronobiology International* 2008; 25 (4): 533–547
- [10] Affrime M, Gupta S, Banfield C et al. A pharmacokinetic profile of desloratadine in healthy adults, including elderly. *Clinical Pharmacokinetics* 2002; 41 (1): 13–19
- [11] Sun C, Li Q, Pan L et al. Development of a highly sensitive LC-MS/MS method for simultaneous determination of rupatadine and its two active metabolites in human plasma: Application to a clinical pharmacokinetic study. *Journal of Pharmaceutical & Biomedical Analysis* 2015; 111: 163–168
- [12] Muppavarapu R, Guttikar S, Kamarajan K. LC-MS/MS method for the simultaneous determination of desloratadine and its metabolite 3-hydroxy desloratadine in human plasma. *International Journal of Pharmacy and Biological Sciences* 2014; 4 (2): 151–161
- [13] Wang T, Zhang K, Li T et al. Prevalence of Desloratadine Slow-metabolizer Phenotype and Food-dependent Pharmacokinetics of Desloratadine in Healthy Chinese Volunteers. *Clinical Drug Investigation* 2015; 35 (12): 807–813
- [14] Xu HR, Li XN, Chen WL et al. Simultaneous determination of desloratadine and its active metabolite 3-hydroxydesloratadine in human plasma by LC/MS/MS and its application to pharmacokinetics and bioequivalence. *Journal of Pharmaceutical & Biomedical Analysis* 2007; 45 (4): 659–666
- [15] Yang L, Clement RP, Kantesaria B et al. Validation of a sensitive and automated 96-well solid-phase extraction liquid chromatography-tandem mass spectrometry method for the determination of desloratadine and 3-hydroxydesloratadine in human plasma. *Journal of Chromatography B* 2003; 792 (2): 229–240
- [16] EMA. Guideline on bioanalytical method validation [www.ema.europa.eu/](http://www.ema.europa.eu/)
- [17] Crowe A, Wright C. The impact of P-glycoprotein mediated efflux on absorption of 11 sedating and less-sedating antihistamines using Caco-2 monolayers. *Xenobiotica* 2012; 42 (6): 538–549
- [18] Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med*. 1995; 332 (20): 1351–1362
- [19] González-núñez V, Valero A, Mulla J. Safety evaluation of desloratadine in allergic rhinitis. *Expert Opinion on Drug Safety* 2013; 12 (3): 445
- [20] Haria M, Fitton A, Peters DH. Loratadine. A reappraisal of its pharmacological properties and therapeutic use in allergic disorders. *Drugs* 1994; 48 (4): 617–637
- [21] Poluzzi E, Raschi E, Godman B et al. Pro-Arrhythmic Potential of Oral Antihistamines (H1): Combining Adverse Event Reports with Drug Utilization Data across Europe. *Plos One* 2015; 10 (3): e0119551