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RESEARCH ARTICLE

Application of a liquid chromatography–tandem mass spectrometry method to the pharmacokinetics, bioavailability and tissue distribution of neohesperidin dihydrochalcone in rats

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Abstract

1. This study was aimed at developing a high sensitive and selective liquid chromatography–tandem mass spectrometry method to quantify neohesperidin dihydrochalcone (NHDC) in rat plasma and tissues for pharmacokinetic, bioavailability and tissue distribution studies.
2. Biological samples were processed with one-step protein precipitation. Rutin was chosen as the internal standard (IS). Chromatographical separation was achieved on an SB-C_{18} (2.1 mm × 150 mm, 5 μm) column with acetonitrile–0.1% formic acid in water as the mobile phase with gradient elution. Electrospray ionization (ESI) source was applied and operated in negative ion mode; selected ion monitoring mode was used for quantification using target fragment ions *m/z* 611.4 for NHDC and *m/z* 609.1 for IS.
3. Calibration plots were linear over the range of 10–3000 ng/mL for NHDC. Lower limit of quantification (LLOQ) for NHDC was 10 ng/mL. Mean recovery of NHDC from plasma and tissues was better than 80.3%. Coefficient of variation of intra-day and inter-day precision were both less than 15%. The bioavailability of NHDC was 21.8%.
4. In conclusion, a sensitive, simple and specific LC-ESI-MS method for the determination of NHDC in rat biological samples was developed. This developed method is successfully used in the pharmacokinetic and tissue distribution study of NHDC in rats.

Keywords

LC–MS, neohesperidin dihydrochalcone, pharmacokinetic, tissue distribution

History

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Introduction

Neohesperidin dihydrochalcone (NHDC) (Figure 1) is the flavonoid derivative of hydrogenated neohesperidin chalconethe extracted from natural *citrus* plants. In recent years, NHDC has been receiving considerable attention in medical, food and nutritional research. Its characteristics include: sweetness, refreshing taste, aftertaste durable and an excellent effect in shielding against bitterness (Marti et al., 2008; Yeomans et al., 2007). NHDC is also reported to have antioxidation activity (Choi et al., 2007) and inhibiting mammalian alpha-amylase (Kashani-Amin et al., 2013). However, it is reported to have embryotoxicity, teratogenicity and other toxic effects related to high dosage in rats or humans (Lina et al., 1990; Waalkens-Berendsen et al., 2004) and it has become an emerging pollutant in the environment (Gan et al., 2013; Kashani-Amin et al., 2013; Scheurer et al., 2009). Thus, the study of its pharmacokinetics is necessary for further development and application of medical, food and nutritional research.

HPLC–MS is reported to be a suitable instrument for the determination of NHDC in foods and drinks. Zygler and Scheurer et al. reported their methods based on high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS) for foodstuffs (Scheurer et al., 2009; Zygler et al., 2011). Quantitative assay for NHDC in foodstuffs has also been developed by using capillary electrophoresis (Perez-Ruiz et al., 2000). However, to the best of our knowledge, so far there are no reports about determination method of NHDC *in vivo*. In this study, we will describe a validated HPLC–MS method for determination of NHDC in rats plasma and tissues for pharmacokinetic and tissue distribution studies.

Materials and methods

Chemicals and reagents

NHDC (purity >98.0%) was purchased from Chengdu Mansite Pharmaceutical Co. Ltd. (Chengdu, China) and Rutin (purity >98.0%) was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China), LC-grade acetonitrile and

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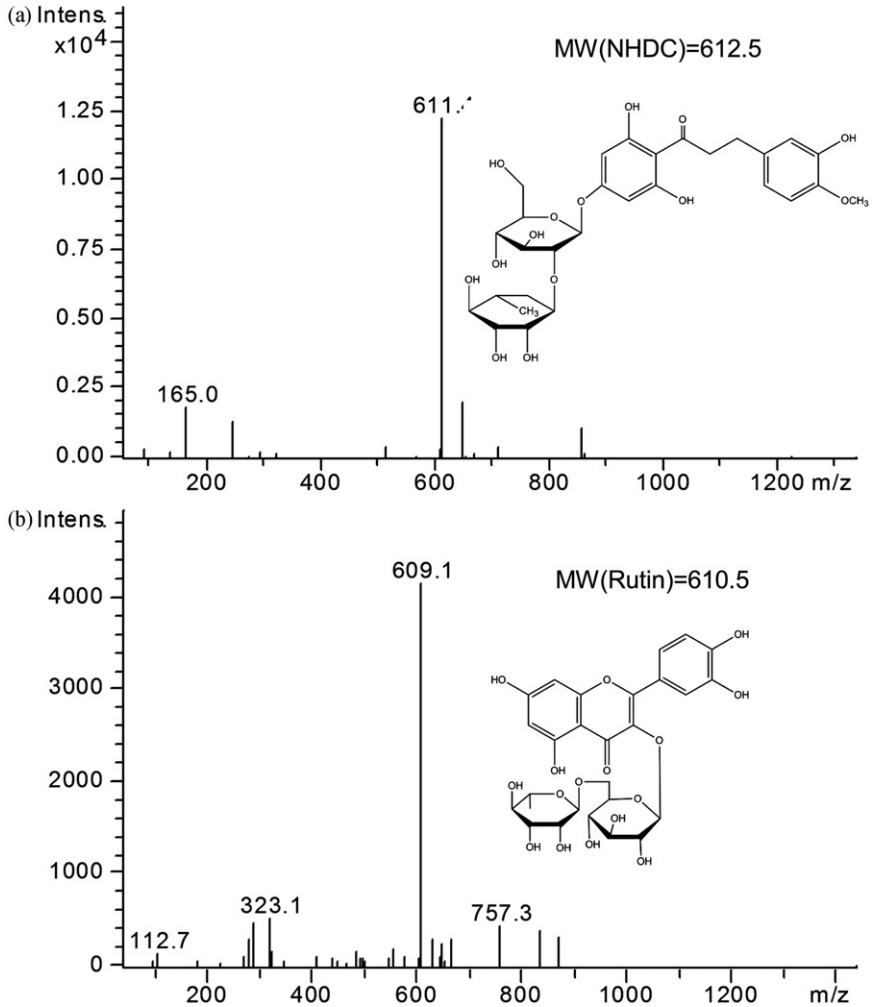

Figure 1. Mass spectra of NHDC (a) and IS (b) in scan mode with ESI (−) source.

methanol were purchased from Merck Company (Darmstadt, Germany). LC-grade formic acid was purchased from Tedia Company (Cincinnati, OH).

Instrumentation and conditions

All analyses were performed with a 1200 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a degasser, an autosampler, a thermostatted column compartment and a Bruker Esquire HCT ion-trap mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source and controlled by ChemStation software (Version B.01.03 [204], Agilent Technologies, Waldbronn, Germany).

Chromatographic separation was achieved on an Agilent Zorbax SB-C18 (2.1 mm × 150 mm, 5 μm) column at 30 °C, with acetonitrile–0.1% formic acid in water as the mobile phase. The flow rate was 0.4 mL/min. A gradient elution program was conducted for chromatographic separation with the mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) as follows: the gradient flow method consisted of an opening condition of 10% solvent B, with a linear increase to 80% solvent B over 4.0 min, then 4.0 min at 80% solvent B, and then a return to the opening condition (10% solvent B) via a linear gradient over 1.0 min, followed by 4.0 min re-equilibration at opening conditions. The total run time was 13 min for each sample.

Drying gas flow (nitrogen) and nebuliser pressure was set at 7 L/min and 30 psi. Dry gas temperature and capillary voltage of the system was adjusted to 350 °C and 3500 V, respectively. Electrospray ionization (ESI) source was applied and operated in negative ion mode. Selected ion monitoring mode was applied to quantify analyte using target ions at *m/z* 611.4 for NHDC and *m/z* 609.1 for internal standard (IS) (Figure 1).

Calibration standards and QC samples

Individual stock solutions of NHDC (1.0 mg/mL) and rutin (IS) (100 μg/mL) were prepared with methanol. Working solutions (0.1, 0.2, 0.5, 2.0, 5.0 10.0 and 30.0 μg/mL) for calibration and controls were prepared from the stock solution by dilution using methanol. Working standard solution of IS (8.0 μg/mL) was prepared by dilution of the IS stock solution with methanol. All of the solutions were stored at 4 °C and brought to room temperature before use.

NHDC calibration standards were prepared by spiking blank rat plasma with appropriate amounts of the working solutions. Calibration plots were constructed in the range of 10–3000 ng/mL for NHDC in rat plasma (concentrations 10, 20, 50, 200, 500, 1000 and 3000 ng/mL). Quality control (QC) samples were prepared as the same way as the calibration standards, three different plasma concentrations (20, 200 and

2000 ng/mL). The analytical standards and QC samples were stored at −20 °C before use.

Sample preparation

Before analysis, the plasma sample was thawed to room temperature. In a 1.5 mL centrifuge tube, an aliquot of 10 μL of the IS working solution (8.0 μg/mL) was added to 100 μL of collected plasma sample followed by the addition of 200 μL acetonitrile. The tubes were mixed by vortex for 0.5 min. After centrifugation at 14 900*g* for 10 min, the supernatant (10 μL) was injected into the LC-ESI-MS system for analysis.

Method validation

Method validation was carried out following FDA guidelines with respect to specificity, recovery, within- and between-day precision, lower limit of detection (LOD), LLOQ and sample stability (US Food and Drug Administration, 2001). Validation runs were conducted on three consecutive days and each validation run consisted of one set of calibration standards and six replicates of QC plasma samples.

The selectivity of this method was evaluated by analyzing blank rat plasma, blank plasma spiked with NHDC and IS, and a rat plasma sample. Calibration curves were constructed by analyzing spiked calibration samples on three separate days. Peak-area ratios of NHDC to IS were plotted against analyte concentrations, and standard curves were closely fitted to the equations by linear regression with a weighting factor of the reciprocal of the concentration ($1/x$) in the concentration range of 10–3000 ng/mL. The lowest standard on the calibration curve should be accepted as the LLOQ. That is to say, NHDC peak (response) should be identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%.

To evaluate the matrix effect (ME), the blank rat plasma was protein precipitated and then spiked with the analyte at 20, 200 and 2000 ng/mL. The corresponding peak areas were then compared with those of neat standard solutions at equivalent concentrations, and this peak-area ratio is defined as the ME. The ME of IS was evaluated at the working concentration (0.8 μg/mL) in the same manner. The recoveries of NHDC at three QC levels ($n = 6$) were determined by comparing peak-area of the analytes in QC samples to the analytes that were added post-protein precipitation at equivalent concentrations. The recovery of the IS was determined in a similar way.

Accuracy and precision were assessed by the determination of QC samples at three concentration levels (20, 200 and 2000 ng/mL) in six replicates for three validation days. The precision was expressed by coefficient of variation (CV). The precision determined at each concentration level should not exceed 15% of the CV.

The stabilities of NHDC in rat plasma were evaluated by analyzing three replicates of plasma samples at the concentrations of 20, 200 and 2000 ng/mL, which were exposed to different conditions. The short-term stability was determined after the exposure of the spiked samples at room temperature for 2 h. The freeze/thaw stability was evaluated after three complete freeze/thaw cycles (−20 °C to 25 °C) on consecutive days. The long-term stability was assessed after storage of the standard spiked plasma samples at −20 °C for 15 days.

Pharmacokinetic study

Male Sprague-Dawley rats (200–220 g) obtained from Laboratory Animal Center of Wenzhou Medical College (Wenzhou, China) were used to study the pharmacokinetics of NHDC. All 12 rats were housed at Wenzhou Medical College Laboratory Animal Research Center. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical College (No: wydw2012-0017) and were in accordance with the Guide for the Care and Use of Laboratory Animals. Animals were housed under controlled conditions (25 ± 1 °C, RH 55 ± 10%) with a natural light–dark cycle. They were allowed to adapt to the housing environment for at least one week before the study. Diet was prohibited for 12 h before the experiment but water was freely available. After fasting for 12 h, six rats were given a dose of 2.0 mg/kg NHDC via the sublingual vein, and the other six rats were administered with the dose of 20 mg/kg NHDC orally. Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0, 0.083, 0.167, 0.333, 0.5, 0.833, 1.333, 2.0, 2.667 and 3.333 h after intravenous administration and at 0, 0.083, 0.167, 0.333, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 24 h after oral administration. The samples were immediately centrifuged at 3000 *g* for 5 min. The plasma obtained (100 μL) was stored at −20 °C until analysis. Plasma NHDC concentration versus time data for each rat was analyzed by DAS software (Version 2.0, China).

Tissue distribution studies

Three groups of male SD rats ($n = 6$ per group) were intravenous administrated at a single dose of 2.0 mg/kg NHDC via the sublingual vein. During the sublingual administration, rats were anesthetized with 10% chloral hydrate. These three groups of rats were euthanized by decapitation at 5 min, 0.5 and 1 h after dosing. Tissues, including the heart, liver, brain, lung, kidney, stomach and spleen were dissected and washed with cold saline. The tissues were then weighed and homogenized in cold saline solution (500 mg/mL). The obtained tissue homogenates were immediately stored at −20 °C until analysis.

Results and discussion

Method development

Various combinations of acetonitrile, methanol, water and formic acid in water with changed content of each component were investigated and compared with identify the optimal mobile phase. Acetonitrile was chosen as the organic phase because it could provide sharper peak shape compared with methanol. Formic acid added into the water could improve the sensitivity, therefore 0.1% formic acid in water was chosen as aqueous phase. Gradient elution with the mobile phase consist of acetonitrile and 0.1% formic acid could provide better peak symmetry, proper retention time and avoided the MEs for the analyte and IS compared with isocratic elution. A flow rate of

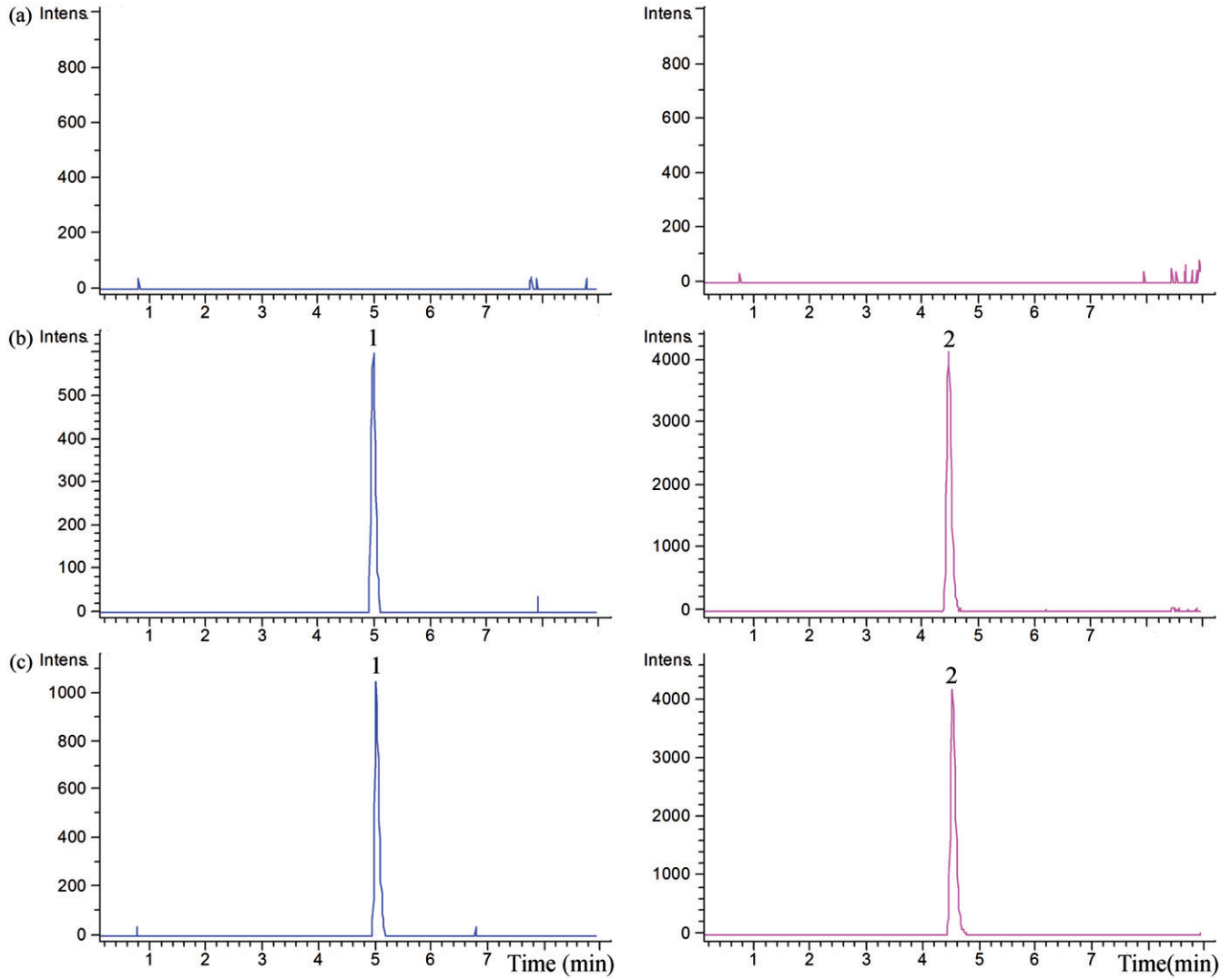

Figure 2. Representative LC-MS chromatograms of NHDC (1) and IS (2): (a) blank plasma; (b) blank plasma spiked with NHDC (20 ng/mL) and IS (800 ng/mL); (c) a rat plasma sample 10 h after oral administration of NHDC (20 mg/kg).

Table 1. Extraction recovery and ME of neohesperidosyl dihydrochalcone in rat plasma and tissues ($n = 6$).

Sample matrix	Concentrations (ng/mL)	Extraction recovery (%)	CV (%)	ME (%)	CV (%)
Plasma	20	85.0	9.0	93.4	8.5
	200	86.3	7.3	100.5	2.2
	2000	86.7	7.7	101.8	8.2
	800 (IS)	82.0	9.4	90.0	8.4
Heart	20	91.9	3.6	106.2	10.0
	200	92.6	4.0	95.3	6.9
	2000	91.6	4.6	94.5	7.0
	800 (IS)	84.7	9.0	92.3	6.1
Liver	20	85.3	8.3	105.7	9.0
	200	85.2	7.5	103.6	8.1
	2000	85.9	8.7	94.3	9.5
	800 (IS)	83.2	8.5	104.1	8.3
Spleen	20	89.3	6.2	97.6	5.3
	200	89.5	5.1	102.7	6.3
	2000	86.0	8.9	104.6	8.2
	800 (IS)	80.8	7.2	97.1	5.6
Lung	20	89.5	5.2	107.8	7.9
	200	97.0	4.8	95.3	6.3
	2000	88.9	9.8	93.4	8.6
	800 (IS)	83.6	6.4	105.2	6.0
Kidney	20	92.0	6.8	92.4	9.3
	200	94.7	3.4	104.3	7.1
	2000	89.0	6.3	107.8	9.2
	800 (IS)	85.8	5.4	104.3	5.2
Brain	20	88.1	14.0	90.5	10.7
	200	93.3	6.3	106.7	4.0
	2000	87.2	8.1	97.3	7.6
	800 (IS)	82.3	8.2	95.7	6.2
Stomach	20	80.3	8.7	107.3	8.9
	200	90.2	5.3	96.0	2.2
	2000	86.2	7.8	95.3	6.2
	800 (IS)	80.7	9.3	94.2	6.9

(*continued*)

Table 1. Continued

0.4 mL/min produced good peak shapes and permitted a run time of 13 min.

An efficient clean-up for bio-samples to remove protein and potential interferences prior to LC–MS analysis was an important point in these studies. The simple and effective protein precipitation was employed in our work. Acetonitrile was chosen as the protein precipitation solvent because it exhibited better effect than methanol, trichloroacetic acid (10%) or perchloricacid (6%), which could provide acceptable recoveries.

Selectivity, recovery and ME

Figure 2 shows the typical chromatograms of a blank plasma sample, a blank plasma sample spiked with NHDC and IS, and a plasma sample. No interfering endogenous substances were observed at the retention times of the analyte and IS. Studies also showed that there were no endogenous interferences in blank tissues.

Mean recoveries of NHDC were better than 85.0% (Table 1). The recovery of the IS was better than 80.7% ($n = 6$). The ME for NHDC in different matrices and between different sources were measured to be ranged from 90.5% to 107.8% at each QC level ($n = 6$), Table 1. The ME for IS (0.8 μg/mL) was in the range of 90.0–105.2% ($n = 6$). As a result, ME from different matrices was negligible in this method.

Calibration curve and sensitivity

The linear regressions of the peak-area ratios versus concentrations were fitted over the concentration range 10–3000 ng/mL for NHDC in rat plasma and different tissues. Typical equation of the calibration curve was shown in Table 2, where y represents the ratios of NHDC peak area to that of IS and x represents the plasma or different tissues concentration. For NHDC, the present LC-ESI-MS method gave an LLOQ of 10 ng/mL with an accuracy of 92.3% and a precision of 15.8% in terms of CV. The LOD, defined as a signal–noise ratio of >3, was 5 ng/mL for NHDC in plasma.

Precision and accuracy

The precision of the method was determined by calculating CV for QCs at three concentration levels over three validation days. Intra-day precision was 12.5% or less and the inter-day precision was 15.0% or less at each QC level (Table 3). The accuracy of the method ranged from 90.0% to 114.0% at each QC level. Assay performance data are presented in Table 3. The results demonstrate that the values are within the acceptable range and the method is accurate and precise for rat biological samples.

Stability

The room temperature, freeze–thaw cycles and long-term (15 days) stability results indicated that the analytes were stable under the storage conditions described above since the bias in concentration was within ±15% of their nominal values, and the established method was suitable for the pharmacokinetic study (Table 4).

Plasma pharmacokinetics

The sensitive and specific LC–MS method of NHDC was applied to the pharmacokinetic study in rat after single dose to 12 rats, including oral (20 mg/kg) and intravenous (2.0 mg/kg) administration. Pharmacokinetic analyses and plasma concentration versus time data were analyzed by DAS software (Version 2.0, Drug Clinical Research Center of Shanghai University of T.C.M and Shanghai BioGuider Medicinal Technology Co., Ltd., China). Pharmacokinetic parameters were calculated by using the non-compartmental model and results are summarized in Table 5. The mean plasma concentration–time curves after single administration of NHDC are shown in Figure 3. As shown in Figure 3, for i.g., the plasma NHDC concentrations increased very quickly within 0.2 h, followed by the gradually decrease in LLOQ by 24 h. The maximum plasma concentration (C_{max}) of NHDC ranged from 801 to 1100 ng/mL, while the half-life ($t_{1/2}$) was 1.0 ± 0.2 h. For i.v., the plasma concentration of NHDC first decreased sharply, and then equilibrium was reached at 3.5 h. The $t_{1/2}$ was 0.4 ± 0.1 h, slightly shorter than that for i.g., revealing that the residence time of NHDC *in vivo* was very short, both for i.g. and i.v. The absolute bioavailability for NHDC was observed to be 21.8% in our study. Pharmacokinetic studies have shown that NHDC could rapidly reach the C_{max} and maintain considerable levels within 2 h.

Tissue distribution

Tissue distribution of NHDC was investigated in rats following a single i.v. dose (2.0 mg/kg). The results (Figure 4) indicated that NHDC underwent a rapid and wide distribution into tissues within the time period examined. It was merely 5 min after administration that NHDC had already reached its C_{max} in most of tissues, including the brain, showing that NHDC could effectively cross the blood–brain barrier. The highest concentration of

Table 2. Regression equation and correlation coefficient of tissues ($n = 6$).

Sample matrix	Regression equation	Correlation coefficient (r)	Linear range (ng/mL)	LLOQ (ng/mL)	LOD (ng/mL)
Plasma	$Y = 0.0014X - 0.0135$	0.997	10–3000	10	5
Heart	$Y = 0.0018X - 0.0638$	0.992	10–3000	10	5
Liver	$Y = 0.0016X - 0.0479$	0.996	10–3000	10	5
Spleen	$Y = 0.0021X - 0.0796$	0.993	10–3000	10	5
Lung	$Y = 0.0008X + 0.0212$	0.992	10–3000	10	5
Kidney	$Y = 0.0019X - 0.0394$	0.996	10–3000	10	5
Brain	$Y = 0.0009X - 0.0014$	0.991	10–3000	10	5
Stomach	$Y = 0.0017X - 0.0184$	0.997	10–3000	10	5

Table 3. Inter-day and intra-day precision and accuracy of neohesperidosyl dihydrochalcone in rat plasma and tissues ($n = 6$).

Sample matrix	Concentrations (ng/mL)	Intra-day		Inter-day	
		Measured concentration (ng/mL)	CV (%)	Measured concentration (ng/mL)	CV (%)
Plasma	20	18.4 ± 2.3	12.5	18.2 ± 1.5	8.2
	200	199.1 ± 10.4	5.2	200.5 ± 10.0	5.0
	2000	1995.9 ± 120.4	6.0	2003.9 ± 155.5	7.8
Heart	20	20.4 ± 2.4	11.8	20.3 ± 1.7	8.4
	200	189.5 ± 4.5	2.4	174.9 ± 13.2	7.5
	2000	1967.2 ± 156.1	7.9	2006.8 ± 154.4	7.7
Liver	20	18.4 ± 1.9	10.3	17.3 ± 2.6	−15.0
	200	185.8 ± 10.6	5.7	190.0 ± 12.9	6.8
	2000	1894.8 ± 171.3	9.0	1903.4 ± 179.4	9.4
Spleen	20	21.7 ± 2.6	11.2	21.8 ± 2.5	11.5
	200	183.4 ± 6.4	3.5	182.2 ± 10.1	5.5
	2000	1950.0 ± 141.3	7.2	2006.3 ± 155.8	7.8
Lung	20	19.4 ± 2.4	12.4	20.6 ± 2.0	9.7
	200	196.4 ± 13.0	6.6	200.3 ± 19.5	8.7
	2000	2037.3 ± 198.0	9.7	1968.7 ± 187.2	9.5
Kidney	20	23.0 ± 2.3	10.0	22.8 ± 2.5	11.0
	200	202.3 ± 15.6	7.7	212.6 ± 19.5	9.2
	2000	2038.1 ± 120.3	5.9	2078.2 ± 140.4	6.7
Brain	20	20.9 ± 2.6	12.4	20.6 ± 1.2	5.8
	200	196.9 ± 11.1	5.6	199.3 ± 13.2	6.6
	2000	1983.2 ± 116.9	5.9	1956.1 ± 196.8	10.1
Stomach	20	18.0 ± 1.9	10.5	19.2 ± 2.3	12.0
	200	197.4 ± 10.6	5.4	203.4 ± 17.1	8.4
	2000	1998.7 ± 190.6	9.5	2014.6 ± 119.1	5.9

Table 4. Stability of neohesperidosyl dihydrochalcone in rat plasma and tissues ($n = 3$).

		Room temperature		Three freeze/thaw cycles		Long term (15 days)	
Sample matrix	Nominal concentration (ng/mL)	Precision CV (%)	Accuracy RE (%)	Precision CV (%)	Accuracy RE (%)	Precision CV (%)	Accuracy RE (%)
Plasma	20	7.9	2.8	1.4	−14.0	6.5	−14.3
	200	6.7	−3.0	14.5	2.1	4.4	2.0
	2000	6.0	−2.5	3.7	−5.5	1.7	1.2
Heart	20	5.1	−3.4	2.7	−5.7	11.9	−9.8
	200	1.0	−0.5	4.0	−1.6	4.3	−8.4
	2000	1.0	−0.1	0.6	−1.0	1.2	−1.3
Liver	20	4.5	−7.0	4.1	−11.4	7.7	0.1
	200	4.5	−7.3	3.7	−6.5	4.3	0.6
	2000	1.1	−1.0	0.5	−0.4	5.4	3.9
Spleen	20	5.8	3.5	6.8	−4.4	4.1	−6.9
	200	3.9	−0.1	2.1	−0.1	2.1	−1.9
	2000	0.8	−1.4	2.1	0.6	1.0	−1.2
Lung	20	4.2	−0.6	7.8	−10.5	6.4	−9.2
	200	1.8	−1.6	3.8	−6.0	5.7	−6.9
	2000	0.6	−1.6	1.3	−0.3	2.3	−0.5
Kidney	20	7.5	3.7	5.1	−4.3	4.1	−6.4
	200	4.2	0.7	2.4	−5.5	4.1	−3.8
	2000	2.0	0.8	3.7	1.0	1.0	−0.7
Brain	20	9.1	−4.4	5.6	−9.1	7.7	−6.2
	200	4.3	1.2	4.4	−4.0	2.3	−5.2
	2000	1.5	−0.4	1.4	−0.5	2.4	1.3
Stomach	20	4.2	−7.1	8.3	−4.6	9.9	−3.6
	200	2.2	−1.1	7.9	−3.1	3.4	−3.5
	2000	1.1	−0.2	1.0	−1.3	1.1	−1.1

NHDC was detected in the heart (1.8 ± 0.5 μg/g), followed by kidneys (1.4 ± 0.28 μg/g) and the liver (1.4 ± 0.4 μg/g), while the lowest concentration of NHDC was also observed in the brain (0.7 ± 0.01 μg/g), followed by the spleen (0.8 ± 0.07 μg/g). The above results showed the absorption of NHDC was fast and could maintain considerable concentrations in most tissues for 1 h after administration. The levels of NHDC in the spleen were much lower than that in other tissues after dosing.

Conclusion

A sensitive, simple and specific LC-ESI-MS method for the determination of NHDC in rat biological samples was developed and validated over the concentration range of

Table 5. Single-compartmental pharmacokinetic parameters for neohesperidosyl dihydrochalcone following oral dose of 20 mg/kg and intravenous administration of 2 mg/kg ($n = 6$).

Pharmacokinetic parameters	Unit	Oral Values (mean ± SD)	I.V. Values (mean ± SD)
Half-life ($t_{1/2}$)	H	1.0 ± 0.2	0.46 ± 0.10
Peak concentration (C_{max})	μg/L	980.3 ± 255.2	2125.9 ± 596.3
Time to peak concentration (T_{max})	h	0.167 ± 0	0.083 ± 0
Area under concentration–time curve (AUC_{0-t})	μg/L × h	2558.7 ± 697.1	1204.5 ± 384.7
$AUC_{0-\infty}$	μg/L × h	2750.6 ± 786.2	1215.2 ± 389.9
Apparent volume of distribution (V)	L/kg	72.9 ± 18.5	1.2 ± 0.49
Clearance (CL)	L/h/kg	7.4 ± 2.5	1.8 ± 0.6
Mean residence time (MRT_{0-t})	h	5.1 ± 0.9	0.57 ± 0.07
$MRT_{0-\infty}$	h	7.2 ± 1.8	0.60 ± 0.08
Bioavailability (F)	%	21.8	

$F = [(AUC_{p.o}) \times (Dose_{i.v})]/[(AUC_{i.v}) \times (Dose_{p.o})] \times 100\%$.

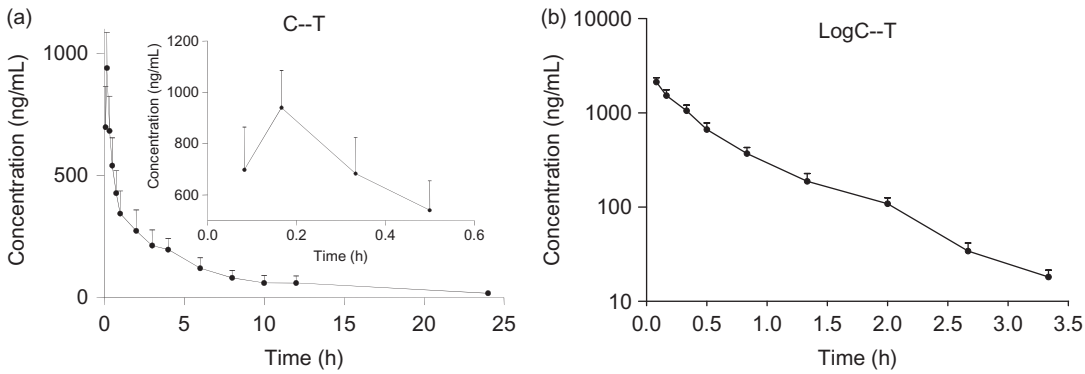

Figure 3. Mean plasma concentration–time curve of neohesperidosyl dihydrochalcone after a single oral dose of 20 mg/kg (a) and mean semi-logarithmic plasma concentration–time curve of intravenous injection of 2 mg/kg (b) to rats. Values are presented as mean ± SD ($n = 6$).

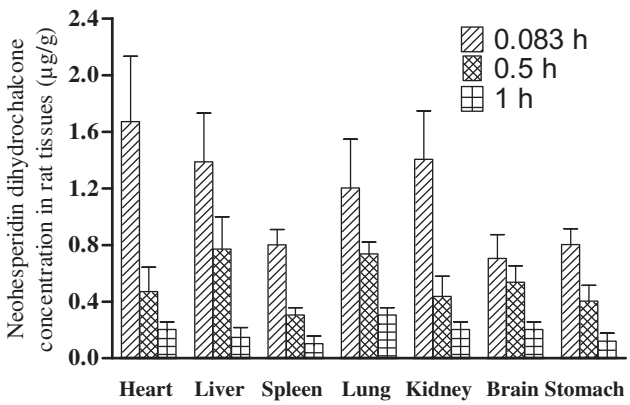

Figure 4. The tissue distribution of NHDC in rats after i.v. administration of a dose of 2 mg/kg.

10–3000 ng/mL. The simple and rapid protein precipitation by acetonitrile was used for pretreatment of plasma samples. This developed method is successfully used in the pharmacokinetic and tissue distribution study of NHDC in rats.

Declarations of interest

The authors report no conflicts of interest.

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