AN ABSTRACT OF THE THESIS OF

Chanida Karnpracha for the degree of Master of Science in Pharmacy presented on May 16, 2013.

Title: Comparison of Pharmacokinetic Data Analysis with Two Competing Pharmacokinetic Software Program

Abstract approved: _____

J. Mark Christensen

Pharmacokinetics (PK) is the study of the transit of drug into, within and removal from the body to reveal how the body acts on a drug when it is taken. There are four major areas in PK: absorption, distribution, metabolism and elimination. Pharmacokinetics research can be separated into two areas: clinical research and analysis of pharmacokinetics values. Upon obtaining drug concentrations at each sample time point, computer analysis using pharmacokinetics software is performed to determine the pharmacokinetics values of the drug: T_{max} (time to peak drug plasma concentration), C_{max} (peak plasma drug concentration), V (volume of distribution), half-life ($t_{1/2}$), elimination constant (k) etc. Current pharmacokinetic software on the market includes programs such as WinNonlin, Kinetica, Pharmod, NONMEM. In this thesis, WinNonlin and Kinetica are used to analyze plasma drug concentrations versus time data sets to compare and validate the results of both software packages. There are two drug models used in this study, xanthohumol and lipoic acid.

Xanthohumol (XN) is the most common flavonoid component found in hops, totaling about 82-89% of the amount of prenylated flavonoids present. However, there are other prenylflavonoids, isoxanthohumol (IX) and 8-prenylnaringenin (8PN). Isoxanthohumol (IX) can occur during the brewing process. The content of xanthohumol

and isoxanthohumol depends on brewing conditions. 8-prenylnaringenin is produced by O-demethylation of isoxanthohumol. In this study xanthohumol was given in 20, 60 or 80 mg doses to healthy volunteers. After xanthohumol administration the plasma concentrations of xanthohumol increased rapidly with all three doses and reached the peak concentrations in 0.78, 1.23 and 2.03 hours for 20, 60 and 180 gm dose of xanthohumol, respectively. Xanthohumol and its metabolites were eliminated in 1, 2 or 3 days. Moreover there was linearity in the pharmacokinetics of xanthohumol as shown in the C_{max} versus dose curve, R^2 is 1. Isoxanthohumol was also formed rapidly from xanthohumol. T_{max} are 7 and 5 hours for medium and high dose respectively. Xanthohumol and isoxanthohumol each have a high volume distribution. Unfortunately, no results for 8-prenylnaringenin (8PN) were obtained due to plasma drug concentrations being undetectable in all subjects.

Lipoic acid (LA) is also known as alpha lipoic acid or thioctic acid. It has two enantiomers, (*R*)-(+)-lipoic acid (RLA) and (*S*)-(-)-lipoic acid (SLA). A racemic mixture (*R*/*S*)-lipoic acid (R/S-LA) is commercially available. R-form of lipoic acid occurs naturally in food but the synthetic product is a racemic mixture. Lipoic 500mg in R- and racemic forms were given to healthy volunteers. Lipoic acid is absorbed rapidly in both forms, 40 minutes for racemic form and 30 minutes for R-form. The C_{max} and AUC values of racemic form are comparable to the R-form, around 2400 ng/mL for Cmax and 115,000 min*(ng/mL) for AUC.

Comparing the results obtained on the pharmacokinetic parameters from the two pharmacokinetic software, Kinetica and WinNonlin revealed that almost all pharmacokinetic parameters of xanthohumol and isoxanthohumol obtained from Kinetica and WinNonlin are the same. However for the pharmacokinetic parameters of lipoic acid, all parameters are different, p-value <0.05, except for C_{max} and T_{max}. The comparisons of pharmacokinetic parameters show that only the normal data set of xanthohumol and isoxanthohumol produced the same results from Kinetica and WinNonlin software programs. The pharmacokinetic results obtained from pharmacokinetic programs vary

due to the different methods of calculation and variations and limitations of the two programs.

Comparison of Pharmacokinetic Data Analysis with Two Competing Pharmacokinetic Software Program

by

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APPROVED:

Major Professor, representing Pharmacy

Dean of the College of Pharmacy

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Chanida Karnpracha, Author

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CHAPTER 1

INTRODUCTION

Pharmacokinetics (PK) is the study of the transit of drug into, within and removal from the body to reveal how the body acts on a drug when it is taken. There are four major areas in PK: absorption, distribution, metabolism and elimination. Absorption is the process of drug movement from the site of drug administration to blood circulation. The barriers in this action vary due to the unique properties that exist in skin, buccal cavity, stomach or intestine. After getting into blood, the drug will be transported via the blood to various areas of the body like adipose tissue, muscle tissue, bone or brain. This process is called distribution. The chemical properties of drugs are important in this process. For example, if the drugs are lipophilic and in unionized form, they will be likely distributed mainly to lipid tissue. The third area of PK is metabolism. The body produces many enzymes that help break down and transform the potent drugs to more hydrophilic substances that can be easily eliminated by liver or kidney, the elimination process. However, some substances can be excreted by tear, sweat, breath or saliva.

Pharmacokinetics research can be separated into two areas: clinical research and quantitative analysis of pharmacokinetics values. In clinical research, the designed drug is administered to animals or human subjects, either, healthy or patient. Blood samples are taken and used to measure the content of drug in our body to assess the therapeutic status of the patient on a drug but sometimes urine is also taken and used. There are many techniques that measure drug concentration in a sample such as HPLC, UPLC or LCMS.

Upon obtaining drug concentrations at each sample time point, computer analysis using pharmacokinetic software is performed to determine the pharmacokinetic values of the drug: T_{max}, C_{max}, V, half-life ($t_{1/2}$), elimination constant (k) etc.

Today, there is a lot of pharmacokinetic software on the market such as WinNonlin, Kinetica, Pharmod, NONMEM. Many companies and some organizations try to develop software program in order to support the clinician or analyst. The programs vary in features and range of medications supported.

In this thesis, WinNonlin and Kinetica are used to analyze drug concentrations versus time data sets to compare and validate the results of both software packages.

WinNonlin is currently the most popular software for pharmacokinetic data analysis. It was released in 1984 by the Pharsight company. It has been used by FDA since 1998 and there are many commercial and academic users worldwide. However, the problem of this program is the huge cost of licensing this software especially for the academic users.

Kinetica is a pharmacokinetic, pharmacodynamic, and noncompartmental data analysis software application and was first released by ThermoScientific. Kinetica improves the data analysis experience while reducing the need for multiple software packages and associated training.

There are two data sets in this thesis and separated in two chapters. Chapter 2 is the data set of Xanthohumol and chapter 3 is the set of Lipoic acid.

CHAPTER 2

XANTHOHUMOL

Chanida Karnpracha, J. Mark Christensen

INTRODUCTION

Flavonoids are the polyphenolic compounds that are most commonly found in fruits, vegetables and flowers. Over 4000 different flavonoids in nature have been described. There are many subgroups of flavonoids: flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids. In the past twenty years, many studies described the wide range of beneficial activities of flavonoids both biological and pharmacological actions including antiviral, anti-inflammatory, antioxidant and anticancer.[4]

Hops (*Humulus lupulus* L.) have been known widely as brewing material, bitterness and flavor. The female inflorescences are used in the beer industry. Xanthohumol (XN) is the most common flavonoid component found in hops, about 82-89% of the total amount of prenylated flavonoids present.[5] However there are other prenylflavonoids, isoxanthohumol (IX) and 8-prenylnaringenin (8PN). Prenylated flavonoids may be divided into two groups: prenylated chalcones (xanthohumol) and prenylated flavanones (isoxanthohumol and 8-prenylnaringenin). All three compounds show activity as inhibitors of CYP1A2 pointing to a potential preventive substance for cancer.[6] In 1999, a study showed that xanthohumol (XN) and isoxanthohumol (IX) had antiproliferative and cytotoxic effects against breast and ovarian cancer in humans.[7] Xanthohumol has also been patented as an osteoporosis drug in 1997.

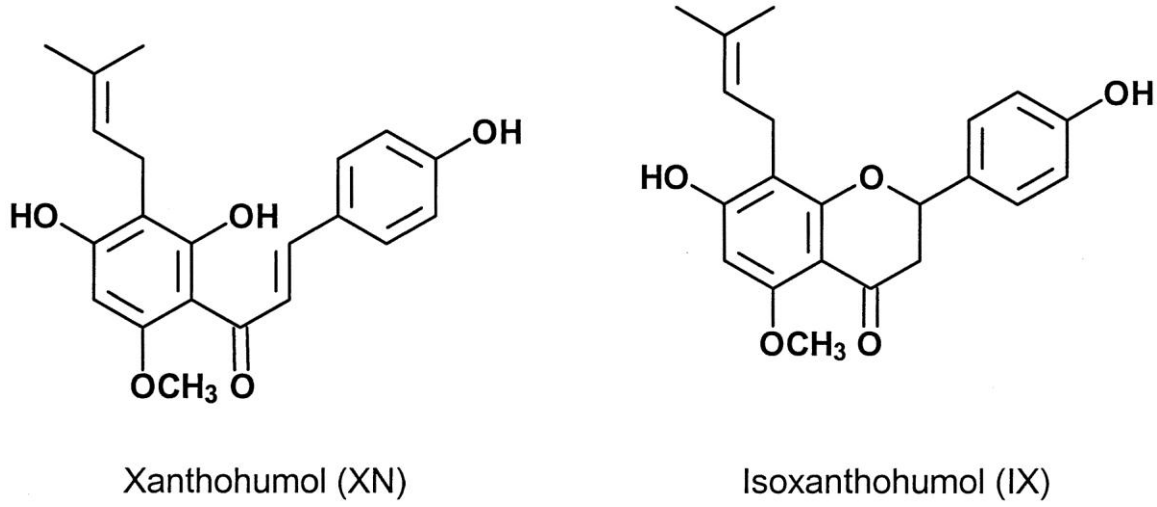


Figure 1 Chemical structure of Xanthohumol and Isoxanthohumol [8]

Moreover hops is also used as an alternative medication for alleviate menopause-associated symptoms.[9] Estrogenic activities in hops have been studied for many years. Both isoxanthohumol and 8-prenylnaringenin have this effect but 8-prinylnaringenin (8PN) exhibits stronger estrogenic potency than the other compounds. Moreover in 2003 there was a study indicating that 8PN and other environmental estrogens (genistein (Gen) and nonylphenol (NP)) can significantly stimulate mammalian sperm function with the environmental estrogens being much more potent than 17beta-estradiol. These responses suggest that classical estrogen receptors may not be involved. [10, 42]

Xanthohumol is very soluble in ethanol, nearly insoluble in water. Xanthohumol is mainly metabolized in the liver and colon. O-methylation, sulfation and glucuronidation of hydroxyl groups occur in liver. In the colon, bacteria play an important role in the ring fission of flavonoids.[4] An in vitro biotransformation study in rat liver indicated that the CYP1A family are the main enzymes for hydroxylation and cytochrome P450 is involved in flavonoid demethylation.[11] There were three main polar metabolites produced in liver microsomes in either treated or untreated rats with various P450 inducing agents. Nevertheless in some groups other nonpolar metabolites were also formed.[12] Xanthohumol also undergoes phase II biotransformation by glucuronidation and sulfation processes by β-glucuronidase,

UDP-glucuronosyltransferases and sulfotransferase in the liver and the gastrointestinal tract.[13,14]

Isoxanthohumol (IX) can occur during the brewing process. The content of xanthohumol and isoxanthohumol depends on brewing conditions.[15] An in vitro study showed that isoxanthohumol was formed from xanthohumol by acid-catalyzed cyclization in the stomach. This reaction followed first-order kinetics with a half-life 37 minutes. Isoxanthohumol was further oxidized on the prenyl side-chain like xanthohumol but forms in both *trans*- and *cis*-alcohol whereas xanthohumol was converted to only *trans*-form.[16]

8-prenylnaringenin was produced by O-demethylation of isoxanthohumol in human liver microsomes and the enzyme CYP1A2 catalyzes this pathway.[16,17] Moreover some studies indicated that the intestinal microbial community plays a crucial role in this conversion therefore interindividual intestinal bacteria colony differences affect this transformation. The selective intestinal bacterium *E. limosum* can convert all IX into 8PN.[18] This process proceeds too slowly according to the results of urinary 8PN excretion from beer consumption in that it took up to several days to occur.[19] Isoxanthohumol was mainly converted to 8PN in the distal colon with up to 80% of conversion occurring there after the absorption and enterohepatic circulation of xanthohumol. The total time needed to produce 8PN is up to 48 hours.[20]

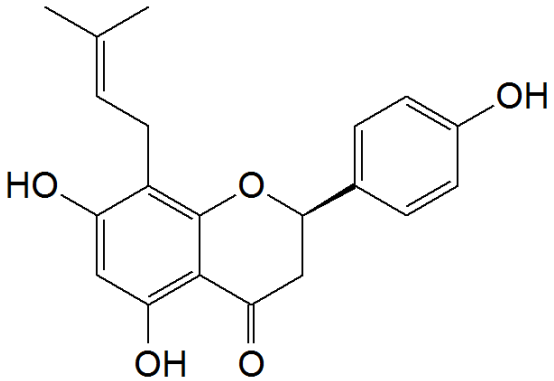


Figure 2 Chemical structure of 8-Prenylnaringenin (8PN)

In 2003 the first in vivo biotransformation study was conducted to determine xanthohumol metabolites in feces of rats. Most of the flavonoid was detected in unchanged form (xanthohumol), approximately 89% and 11% metabolites.[21] The absorption of xanthohumol was very poor after oral administration. Xanthohumol was excreted mainly in feces within 24 hours in both iv. or oral doses. There was no pharmacokinetic difference between extract and pure compound.[22] A Caco-2 intestinal epithelial cell study indicated that xanthohumol did not use a facilitated transporter during absorption. However xanthohumol had specific binding to cytosolic proteins in the cytosol of intestinal cells therefore contributing to poor bioavailability of xanthohumol.[23] In addition, xanthohumol can also be trapped in liver cells such as hepatocellular carcinoma cells (HuH-7) and hepatic stellate cells (HSC).[24]

There are a lot of studies on xanthohumol and its metabolites but until recently no research was published on its absorption, distribution, metabolism and excretion. In 2011, a pharmacokinetic study in rat was performed where xanthohumol was given orally and intravenously. In the iv group the clearance was low and the volume of distribution was high indicating that xanthohumol distributes extensively into tissue. In oral administration group the plasma concentration time curve revealed that there were two peaks one between 0.5 and 2 hours and another between 8 and 12 hours. These results support that xanthohumol can be absorbed in both small and large intestines. Moreover the high peak concentrations of xanthohumol, isoxanthohumol, 8-prenylnaringenin and 6-prenylnaringenin are related to the biotransformation process.

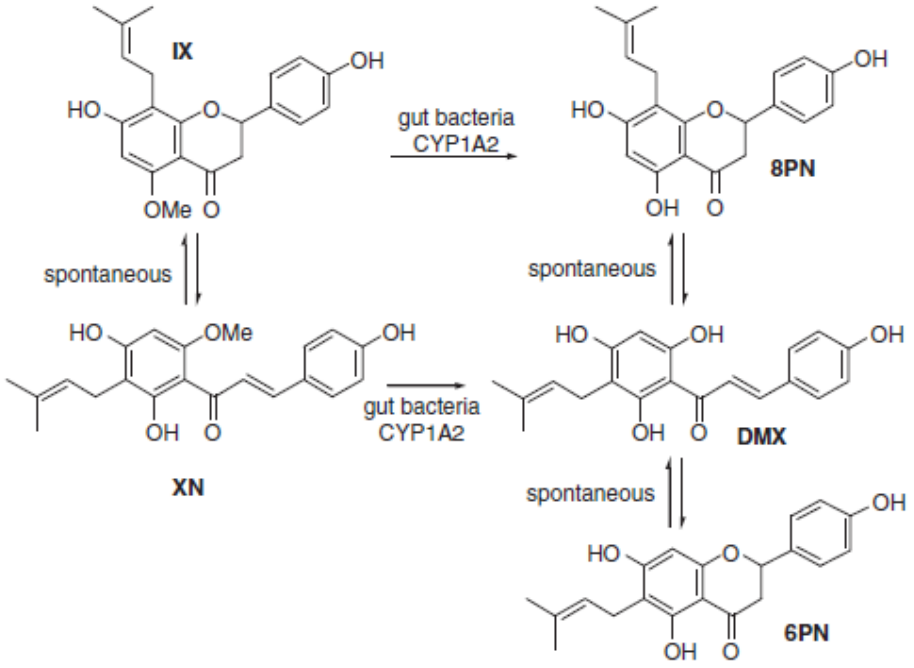


Figure 3 The biotransformation of xanthohumol and its metabolites.[25]

The bioavailability of total xanthohumol was 33.1, 13.4 and 10.8% for low, medium and high dose, respectively. The reason that the medium and high dose had lower bioavailability than the low dose may be due to the low aqueous solubility of xanthohumol. An optimized dosage formulation should be developed to solve this problem. [25]

This research the aim was to study the pharmacokinetics of xanthohumol in human by using data from a previous study. Xanthohumol was given to healthy volunteers divided into three groups 20 (low dose), 60 (medium dose) and 180 (high dose) mg. The amount of dose given was calculated from the previous study in rat. The formulation is a capsule containing a microemulsion of xanthohumol in order to improve the solubility and bioavailability of drug. Additionally, a comparison of the results obtained of fitted pharmacokinetic parameters from two pharmacokinetic software packages (Kinetica version 5.0 and WinNonlin version 5.3) using paired t-tests in R program was performed.

MATERIALS AND METHODS

PHARMACOKINETICS SOFTWARE

There were two pharmacokinetic software programs used in this study, Kinetica version 5.0 (Thermo Scientific) and WinNonlin version 5.3 (Pharsight Cormporation).

Kinetica was the pharmacokinetic-pharmacodynamic (PK/PD) template and method-driven system developed by Thermo Scientific. There are both default validated methods, more than 50 different methods and customized methods. The validated methods can be saved as the template and used for other analysis to provide a consistency during analyses and for the analysts. The templates and methods in Kinetica are made in only one interface including a series of panes, spreadsheets, graphical and web views. Moreover in bioequivalence studies, Kinetica provides the statistics package that meets the requirements of the FDA, EMEA and MHW regulatory guidelines. Kinetica offers fast, high-throughput data analysis for discovery, preclinical, clinical, drug metabolism and drug delivery settings.

Kinetica is installed with nine subdirectories:

- Absorption Kinetics
- Compartmental Fitting
- Convolution/Deconvolution
- Enzyme Kinematics
- In Vivo/In Vitro Correlation
- Non-Compartmental Analyses
- Population Pharmacokinetics
- Protein Binding
- Urine Pharmacokinetics

WinNonlin is distributed by Pharsight Corporation. There are compartmental and noncompartmental models in order to analyze the raw data from pharmacokinetic and pharmacology studies. In addition, the user can also develop templates and save them as user models for use next time. WinNonlin provides a large library of pharmacokinetic, pharmacodynamic, noncompartmental, PK/PD links, and indirect response models.

WinNonlin is a tool for non-linear modeling. It can be programmed to fit differential equations, algebraic equations or the combination of both in pharmacokinetic modeling. WinNonlin will not provide the results that show the correctness but it shows how good the model fits the data. Least squares is used in this program to fit drug concentrations versus time data. Moreover, within WinNonlin a descriptive statistic package provides mean, mode, median, variance and standard deviation.

METHODS

Noncompartmental analysis was performed on the xanthohumol concentrations versus time data using both Kinetica and WinNonlin software. Noncompartmental analysis is performed when compartmental analysis can not specify whether a one, two or three compartment model can fit the data. Noncompartmental analysis uses the area under the curve (AUC) to estimate the exposure of drug.

AUC is calculated by using the trapezoidal rules.

$$AUC = \frac{1}{2}(C_1 + C_2)(t_2 - t_1)$$

* C is the plasma drug concentration and t is the sampling time point.

In Kinetica software, there are three types of calculation:

- Linear: computes all values in a linear manner.
- Log linear: computes all values prior to C_{max} in a linear manner and transforms all values after C_{max} to log

in its computation.

- Mixed log linear: the computation method where all ascending phases are computed in a linear manner and all descending phases are computed based on their log values.

For WinNonlin, there are four calculation methods of AUC as following:

- Linear/log trapezoidal: log-trapezoidal rule, using linear interpolation between log-transformed data through C_{last}.
- Linear trapezoidal (linear interpolate): linear trapezoidal rule, using linear interpolation between untransformed data through C_{last}
- Linear up/log down: linear trapezoidal rule up to C_{max}, log-trapezoidal rule after C_{max}; uses linear interpolation between untransformed data up to C_{max}, log-transformed data from C_{max} through C_{last}.
- Linear trapezoidal (linear/log interpolation): trapezoidal rule with linear interpolation between data points up to C_{max}, log-linear interpolation between data points from C_{max}, extrapolated to T_{inf}.

In this study we choose the mixed log linear for Kinetica and linear up/log down for WinNonlin. These two options are more accurate than other linear or log trapezoidal combination or used alone. Linear trapezoidal is the best approximation in absorption phase, increasing part. On the other hand logarithmic trapezoidal is fitted to the elimination phase, decreasing area. Log trapezoidal AUC is calculated by this following equation:

$$AUC = \frac{C_1 - C_2}{\ln(C_1) - \ln(C_2)}(t_2 - t_1)$$

In WinNonlin, uniform weighing of the data was used on the plasma concentrations collected over time being fitted. However there was no choice in Kinetica software where uniform weighting of the data is only available.

PHARMACOKINETIC PARAMETER

The elimination constant (k) was calculated by the terminal slope during the elimination phase in each subject using the regression data analysis in Microsoft office Excel 2007. AUC and AUMC are calculated by the trapezoidal rule. MRT (mean residence time) was determined by using the following equation:

$$MRT = AUMC/AUC$$

The other parameters were calculated by using these following equations:

$$t_{1/2} = 0.693/k$$

$$Cl/F = dose/AUC$$

$$V_{ss}/F = Cl*MRT$$

* F is bioavailability of drug.

MODEL DRUG

Xanthohumol (XN) capsules used contained either 20, 60, or 180 mg of xanthohumol. However, this formulation used a microemulsion of the drug inside the capsule instead of powder or granules. Due to the poor solubility of xanthohumol, a microemulsion was used to solve this problem and improve bioavailability and absorption.

Preparation utilizing microemulsion is a valuable approach in constructing drug formulations. It provides high drug solubility, transparency, thermodynamic stability and high diffusion and absorption rates. The main composition is comprised of an oil phase, water phase, surfactant and co-surfactant in order to blend these two phases together and

make a transparent emulsion. Microemulsions are used in drug dosage forms for many routes of administration such as oral, nasal, dermal, transdermal, ocular, vaginal, rectal, buccal and parenteral. This formulation can carry either hydrophilic or lipophilic drugs depending on the dosage form. If the drug is water soluble, a water in oil formulation would be preferred.[31,32] Microemulsions differ from an emulsion by its smaller size. The smaller micellar size increases the surface area that provides higher drug solubility.

Xanthohumol given to the human volunteers was in powder form that was dissolved into an isotropic mixture of oleic acid, Tween 80 and propylene glycol to promote maximum absorption. Capsules were filled with this microemulsion at doses of 20, 60 and 180mg of xanthohumol.

ANALYZING COMPONENTS

Xanthohumol and its metabolites (isoxanthohumol (IX) and 8-prenylnaringenin (8PN)) were measured by using LCMS.[25]

SUBJECTS

A study was conducted using healthy volunteers (n=18, 13 and 17) where Xanthohumol 20, 60 and 180mg doses were given. Each subject took one capsule of the respective dose.

STATISTICS

The comparison between the pharmacokinetic results obtained from Kinetica and WinNonlin programs was performed using a paired t-test in R program. A probability level of 0.05 or less was considered to indicate statistical significance.

RESULTS

XANTHOHUMOL

The mean plasma concentrations versus time curves after oral administration of xanthohumol are shown in Figures 4.1, 5.1 and 6.1. The semilogarithmic plots of plasma concentrations versus time after administration of xanthohumol are shown in Figures 4.2, 5.2 and 6.2.

From the curves, after xanthohumol administration the concentrations increased rapidly with all three doses and reached the peak concentrations in 0.78, 1.23 and 2.03 hours for 20, 60 and 180 gm of xanthohumol, respectively. A second concentration peak was reached a couple hours after the first, around 4 hours after administration. The second peak can be observed clearly in the low dose and high dose data. For the medium dose, a plot of mean plasma concentration vs. time data reveals there is only one peak. However in individual plots most subjects show a little second peak at about 4 hours. The concentrations declined after the second peak and all of the xanthohumol and its metabolites were eliminated in 1, 2 or 3 days depending on dose of drug with the high dose taking a longer time for elimination than for the smaller doses.

The PK parameters obtained from Kinetica software for the three doses are listed in Tables 1, 2 and 3. The PK values of subject no. 2, 4, 13 and 14 in low dose (20 mg) were calculated by hand because the program could not fit these four subjects' data.

For the mean values of each pharmacokinetic parameter in low dose, subjects' number 13 and 14 were excluded because there were not enough data for calculation.

The PK parameters obtained from WinNonlin software for the three doses are listed in Tables 4, 5 and 6. The PK parameter values of subjects' number 4 and 13 in low dose (20 mg) were calculated by hand because the program could not fit the data from these subjects. The same manual results from the calculation were used for both Kinetica

and WinNonlin. For the mean value of each pharmacokinetic parameter in the low dose, subjects' number 13 and 14 were excluded because there were not enough data for calculation.

The C_{max} was around 45, 66 and 133 ug/L and T_{max} was 2, 10 and 17 hours for low, medium and high dose respectively. C_{max} is the highest drug concentration after administration whereas T_{max} is the time to reach C_{max}. Xanthohumol was absorbed very fast in low dose yielding the lowest T_{max}. For the elimination phase, xanthohumol was excreted yielding an elimination rate constant (k) of 0.333, 0.067 and 0.041hr^{-1} in low, medium and high dose groups. Xanthohumol had a high volume distribution in all three groups.

In Figure 7, a plot of C_{max} versus dose and AUC versus dose show the linearity of the pharmacokinetics of Xanthohumol, R^2 is 1. That means drug concentration at steady state will change in proportion to the dose. When the dose is increased, the C_{max} and AUC will increase in the same proportion.

A comparison results between the two programs shows all pharmacokinetic parameters in all three dosage groups are not different. Kinetica and WinNonlin appear to provide similar results.

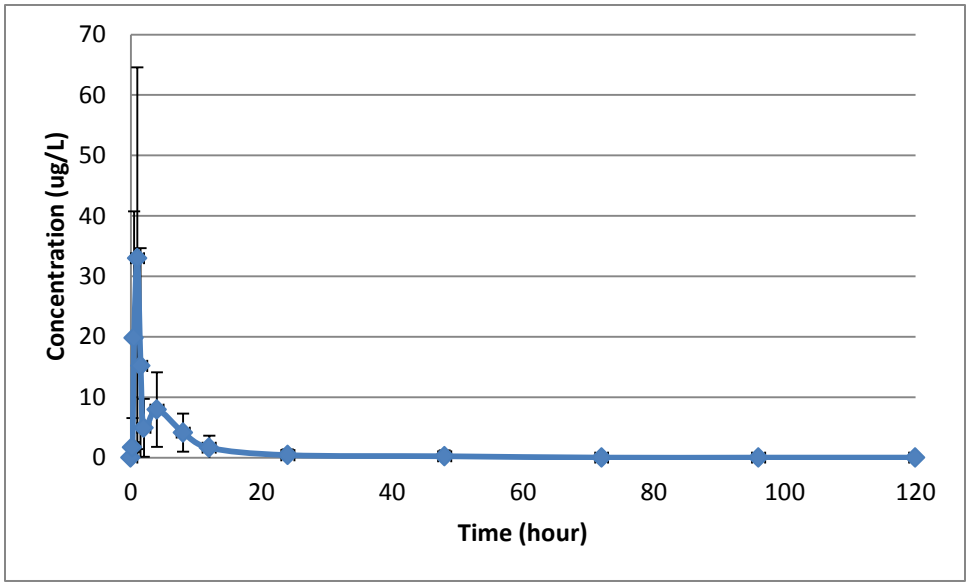


Figure 4.1 Plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 20mg

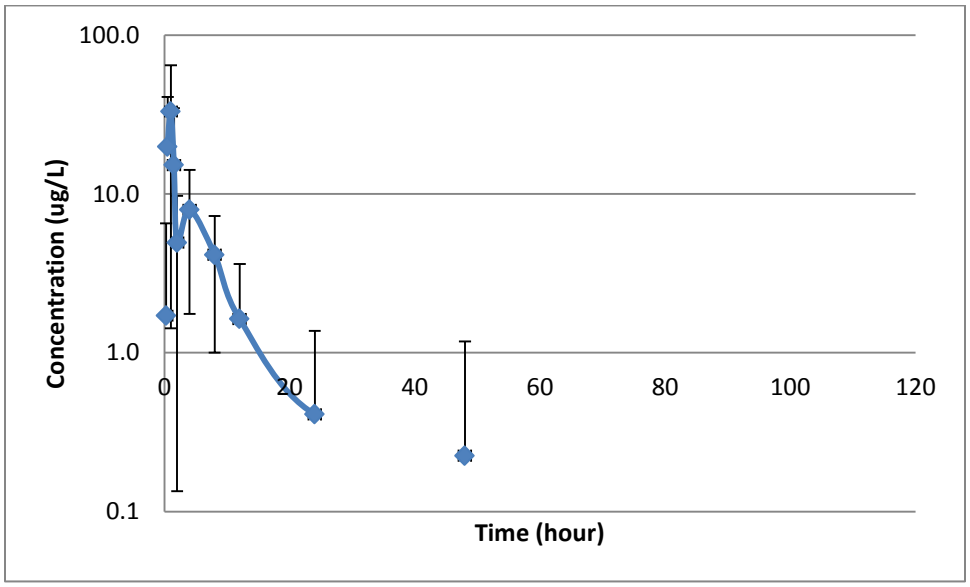


Figure 4.2 Semilogarithmic plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 20mg

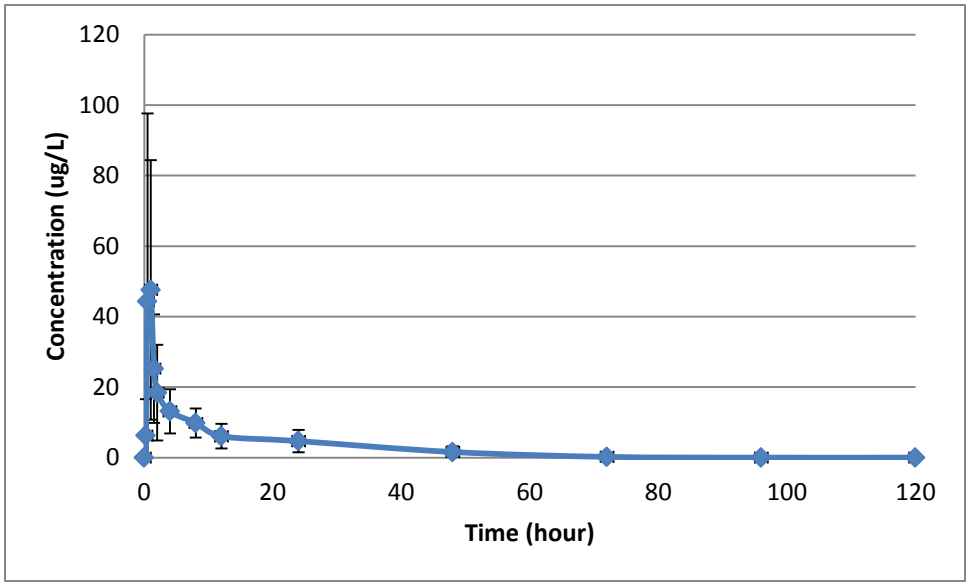


Figure 5.1 Plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 60mg

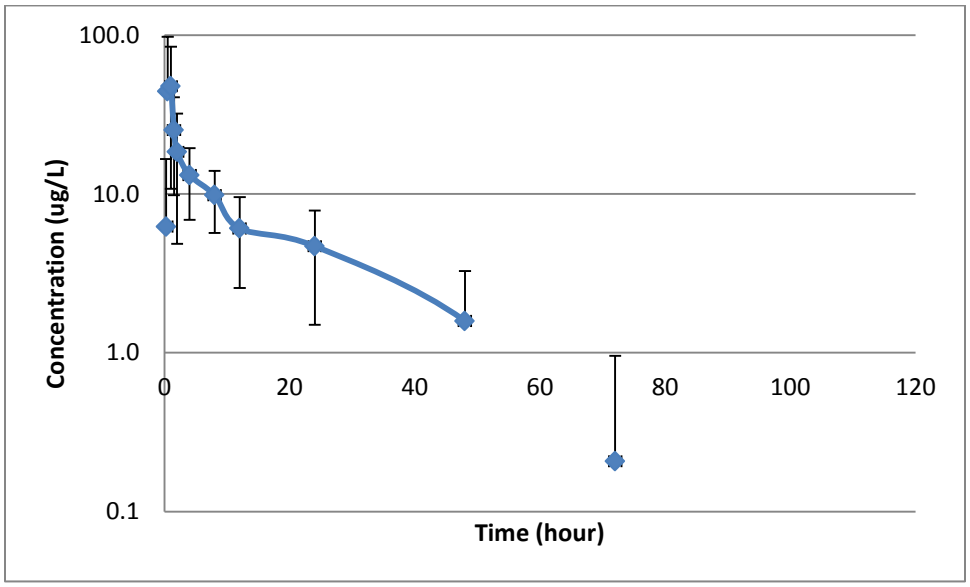


Figure 5.2 Semilogarithmic plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 60mg

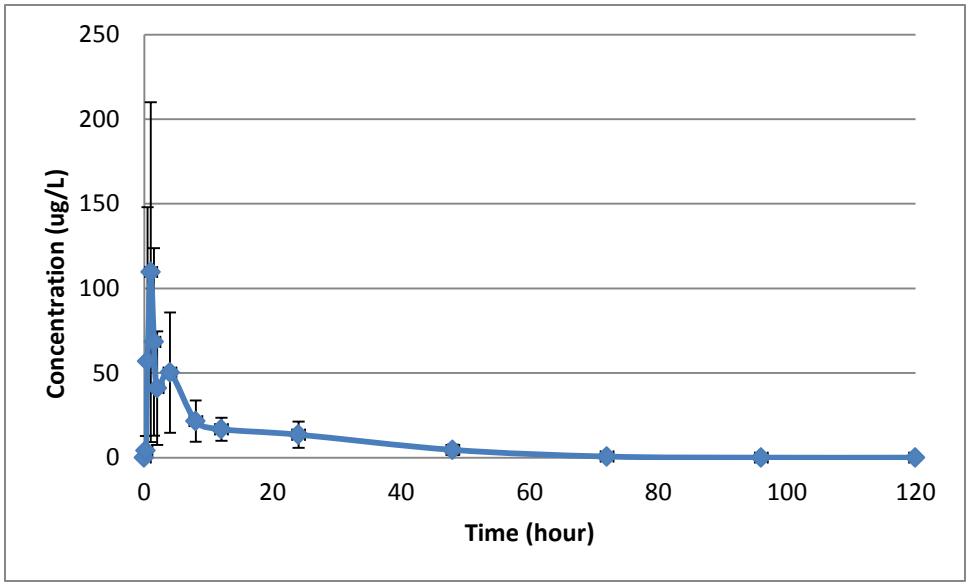


Figure 6.1 Plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 180mg

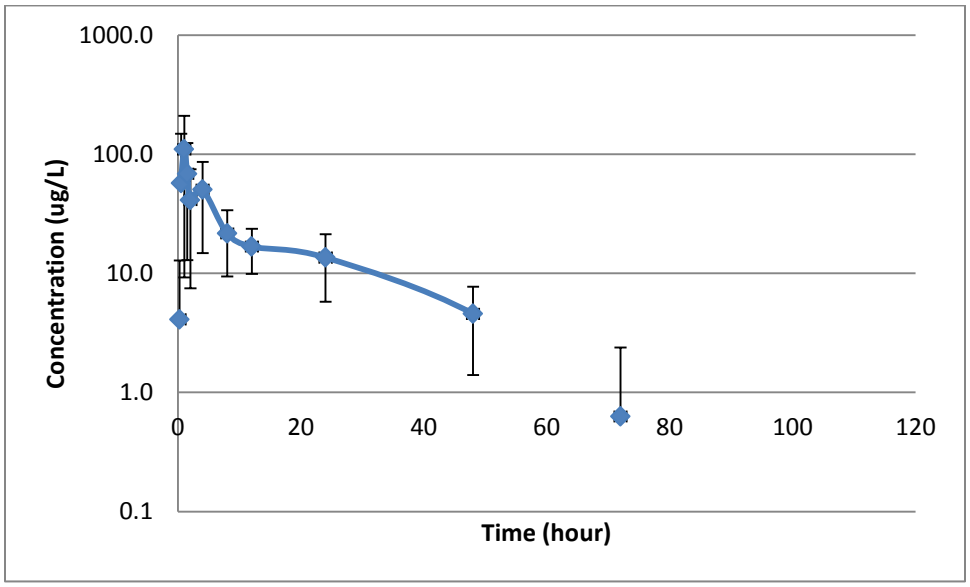


Figure 6.2 Semilogarithmic plot of mean plasma concentrations of Xanthohumol vs. time after administration of Xanthohumol 180mg

Table 1 The Pharmacokinetic parameters from Kinetica software of 20 mg of Xanthohumol

Sample	Parameter								
	C_{max} (µg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(µg/L))	AUMC (h^2*(µg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	33.5061	1.0000	5.8759	0.1180	66.7929	942.0182	12.0655	256.1624	2171.5084
2	115.4040	1.0000	28.5773	0.0243	317.6148	6608.2705	20.8583	63.1279	1316.7425
3	19.4936	0.5000	7.7049	0.0900	78.8800	1021.3918	10.9806	215.0121	2390.0467
4	20.7090	1.0000	0.2090	3.3165	11.4098	12.7360	1.1195	1757.9327	1967.9204
5	83.7000	1.0000	4.0362	0.1717	177.0388	1270.5930	7.2262	113.7453	662.3343
6	76.5000	1.0000	2.7184	0.2550	182.3976	972.9939	4.8468	99.6258	390.7170
7	71.4500	0.5000	5.1285	0.1352	138.9690	1105.5408	6.3505	114.8842	850.0076
8	69.5500	1.5000	2.2708	0.3052	181.3794	791.5434	4.4841	113.3005	371.1818
9	28.5147	0.5000	15.0277	0.0461	124.8860	2569.8431	18.1299	141.0972	3059.0484
10	21.1692	0.5000	2.1352	0.3246	26.0277	123.6379	4.0181	649.9819	2002.1866
11	30.5679	0.5000	10.3270	0.0671	53.2099	1325.5696	13.5605	204.5986	3048.2431
12	29.5059	1.0000	10.7930	0.0642	54.3581	1361.8120	13.7406	201.7989	3142.2067
13	4.2787	1.5000	3.8289	0.1810	10.0843	28.5000	3.0000	2105.2632	6315.7895
14	11.7351	1.0000	5.6438	0.1228	47.9962	295.8605	6.6925	452.4068	3027.7168
15	12.9741	0.5000	6.0240	0.1151	39.4614	586.4514	9.7229	331.5851	2881.7335
16	21.0984	0.5000	18.9234	0.0366	136.2941	4720.2107	26.5665	112.5647	3073.0905
17	36.0195	1.0000	4.9679	0.1395	86.6939	964.8305	8.0659	167.1973	1198.3314
18	52.5100	0.5000	6.1363	0.1130	81.5805	689.7487	7.8842	228.6106	2023.8449
mean	45.167	0.781	2.084	0.333	109.812	1566.699	10.601	156.789	1909.321
SD	29.601	0.315	7.329	0.801	78.290	1732.035	6.760	413.125	998.992

* Values were calculated using harmonic mean.

(a) These mean values were excluded sample no. 13 and 14.

Table 2 The Pharmacokinetic parameters from Kinetica software of 60 mg of Xanthohumol

Sample	Parameter								
	C_{max} (µg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(µg/L))	AUMC (h^2*(µg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	48.6750	0.5000	54.1767	0.0128	331.3500	39036.2435	69.6863	107.1101	8371.7728
2	64.0740	1.0000	51.0849	0.0136	331.6261	36968.8512	74.2256	120.4673	8878.4382
3	32.9751	1.0000	22.3576	0.0310	282.2053	10096.8077	31.0934	184.7715	5959.8430
4	49.2060	1.0000	5.9112	0.1173	134.7818	1262.6239	8.6873	412.8200	3520.5323
5	53.2770	2.0000	21.4125	0.0324	625.1817	21836.0022	30.4915	83.7832	2588.2083
6	97.7040	0.5000	12.7073	0.0545	293.3745	5695.7840	17.6236	185.6493	3403.4519
7	49.0290	1.5000	19.9180	0.0348	301.1631	8864.3956	26.4475	179.0141	5144.0711
8	164.4920	0.5000	15.9431	0.0435	632.4361	13554.1160	20.3187	89.9447	2068.8248
9	29.7006	1.5000	6.1443	0.1128	190.3011	1860.4327	10.2238	329.7237	2922.8059
10	68.9120	1.0000	5.8229	0.1190	263.0780	2543.6945	10.2053	240.7190	2022.1977
11	82.3050	0.5000	12.7075	0.0545	263.5563	4752.8153	16.4393	207.5307	3804.6668
12	114.5190	1.0000	19.0906	0.0363	436.8216	10494.2709	22.1327	126.5418	3485.2042
13	14.4963	4.0000	3.3156	0.2091	113.4681	681.7059	6.1885	544.6802	2605.4536
mean	66.874	1.231	10.339	0.067	323.026	12126.749	26.443	160.016	4213.498
SD	40.230	0.949	16.147	0.057	160.012	12900.159	21.751	137.236	2256.421

* Values were calculated using harmonic mean.

Table 3 The Pharmacokinetic parameters from Kinetica software of 180 mg of Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	196.6470	1.0000	19.4907	0.0356	916.4590	24149.6147	25.3132	188.6725	5305.3049
2	164.7870	1.0000	16.1626	0.0429	1045.9808	22147.3277	20.1290	163.5964	3814.6961
3	305.5020	0.5000	33.6797	0.0206	965.4304	42981.7409	35.5532	148.8906	7234.5318
4	31.8423	4.0000	18.6926	0.0371	720.5525	25150.5644	30.8446	220.7514	5953.1646
5	89.9160	1.5000	19.2421	0.0360	1583.8412	62344.9600	36.8007	106.2496	2949.5466
6	188.6820	1.0000	12.5988	0.0550	862.4255	14429.7069	16.2977	203.3020	3695.2513
7	205.3200	1.0000	16.0397	0.0432	1066.7322	19017.5640	17.1660	162.4747	3759.7280
8	111.8640	1.0000	18.2625	0.0380	756.7529	18046.5471	22.2495	221.9216	5847.0182
9	29.3820	4.0000	16.0259	0.0433	432.1423	10393.0142	22.6083	391.5606	9053.0953
10	172.5750	1.0000	15.3051	0.0453	760.9732	16444.2768	20.6046	225.5397	4980.0446
11	137.0000	4.0000	15.9157	0.0436	920.5969	18683.2467	19.3564	186.4857	4281.9959
12	97.7040	1.0000	13.9282	0.0498	859.4208	18993.2503	21.8207	206.7958	4155.3990
13	22.4967	8.0000	18.0150	0.0385	402.3563	11488.7713	26.1951	410.4117	10666.6630
14	71.6850	1.5000	14.2783	0.0485	871.6733	18977.0425	20.8927	198.1703	4082.1691
15	18.1248	2.0000	20.6300	0.0336	292.4521	14150.9482	33.3108	423.7134	12610.9129
16	351.5220	1.0000	17.1899	0.0403	1779.6286	29230.7834	15.7553	97.0194	2406.0638
17	70.8000	1.0000	15.7772	0.0439	426.5237	9245.2438	18.8330	366.6686	8345.9882
mean	133.285	2.029	16.953	0.041	862.585	22110.271	23.749	193.813	5831.857
SD	96.836	1.932	4.635	0.008	387.896	13100.512	6.636	102.904	2853.276

* Values were calculated using harmonic mean.

Table 4 The Pharmacokinetic parameters from WinNonlin software of 20 mg of Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	34.0000	1.0000	6.2258	0.1113	71.0204	1105.3174	12.8630	232.7478	2090.5190
2	115.0000	1.0000	28.5859	0.0242	316.8011	19035.9680	43.8855	46.1080	1901.5285
3	19.0000	0.5000	8.0000	0.0866	83.3601	1149.3662	11.4954	200.0305	2308.6641
4	21.0000	1.0000	0.2090	3.3165	11.3760	12.7360	1.1195	1757.9327	1967.9204
5	84.0000	1.0000	4.0870	0.1696	177.1745	1298.8885	7.3613	113.3473	668.3335
6	77.0000	1.0000	2.7855	0.2488	185.4200	1020.2504	4.9621	97.2732	390.9068
7	71.0000	0.5000	5.7580	0.1204	139.9458	1317.8928	7.1588	108.6396	902.4717
8	70.0000	1.5000	2.3583	0.2939	185.4150	828.3005	4.5961	110.9758	377.5689
9	29.0000	0.5000	16.8560	0.0411	131.1147	3225.5149	20.7095	128.4103	3122.6831
10	21.0000	0.5000	1.9261	0.3599	25.1871	102.1391	3.6089	706.6599	1963.6497
11	31.0000	0.5000	7.1459	0.0970	51.4741	705.6419	9.2359	261.7728	2698.7078
12	30.0000	1.0000	7.5987	0.0912	54.0282	772.7076	9.5495	247.1694	2709.6305
13	4.0000	1.5000	3.8289	0.1810	9.5000	28.5000	3.0000	2105.2632	6315.7895
14	12.0000	1.0000	7.4071	0.0936	44.2087	636.7942	11.8847	373.2659	3988.8049
15	13.0000	0.5000	5.7177	0.1212	38.8940	534.3282	9.2700	346.9771	2862.1779
16	21.0000	0.5000	20.4606	0.0339	140.7535	5723.8315	29.6098	103.4614	3054.0177
17	36.0000	1.0000	4.5369	0.1528	85.0728	836.2202	7.4433	178.0225	1165.2329
18	53.0000	0.5000	6.0518	0.1145	79.5132	655.4245	7.7132	235.3636	2054.9255
mean	45.313	0.781	2.060*	0.336	111.034	2395.283	11.911	147.665*	1889.934
SD	29.570	0.315	7.569	0.800	78.842	4647.220	10.960	417.248	928.346

* Values were calculated using harmonic mean.

(a) These mean values were excluded sample no. 13 and 14.

Table 5 The Pharmacokinetic parameters from WinNonlin software of 60 mg of Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	49.0000	0.5000	38.0518	0.0182	319.4740	21580.5273	48.1531	133.8792	7349.5793
2	64.0000	1.0000	115.6522	0.0060	337.9276	128016.7405	159.5264	74.7682	12475.1392
3	33.0000	1.0000	22.6257	0.0306	283.6274	10009.3355	30.8063	184.6657	6027.8613
4	49.0000	1.0000	5.4816	0.1265	136.0465	1171.1513	8.1511	417.5960	3302.4565
5	53.0000	2.0000	22.4678	0.0309	630.9099	23306.1792	31.7964	81.8575	2653.3490
6	98.0000	0.5000	13.1999	0.0525	300.4098	6191.0244	18.4461	178.7698	3404.3959
7	49.0000	1.5000	19.5559	0.0354	305.4590	8752.3839	25.9034	177.5750	5009.9636
8	164.0000	0.5000	15.4853	0.0448	619.0974	12814.1830	19.7105	92.2908	2061.8246
9	30.0000	1.5000	6.4864	0.1069	201.2919	2115.3876	10.9398	310.2933	2903.6808
10	69.0000	1.0000	5.8943	0.1176	267.5999	2636.4211	10.3954	236.5796	2011.7841
11	82.0000	0.5000	12.9037	0.0537	259.8882	4768.4832	16.6525	209.5320	3900.6603
12	115.0000	1.0000	16.7973	0.0413	418.9871	8559.6264	19.3022	135.3016	3278.8082
13	14.0000	4.0000	3.5997	0.1926	113.7745	723.1501	6.4941	538.8199	2798.2559
mean	66.846	1.231	10.516	0.066	322.653	17741.892	31.252	154.029	4398.289
SD	40.222	0.949	29.389	0.054	156.761	33903.983	40.225	136.765	2881.812

* Values were calculated using harmonic mean.

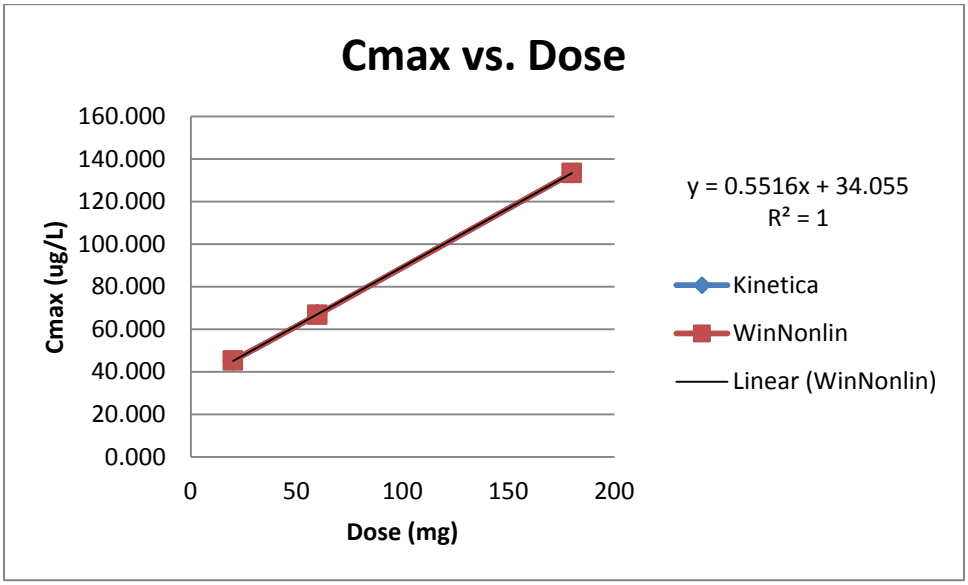
Table 6 The Pharmacokinetic parameters from WinNonlin software of 180 mg of Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	197.0000	1.0000	16.9440	0.0409	907.8775	22044.9715	23.6339	192.9741	4717.2613
2	165.0000	1.0000	16.5437	0.0419	1048.9903	22802.4392	20.5737	162.4068	3876.2527
3	306.0000	0.5000	36.7777	0.0188	980.4282	49611.3204	39.1306	141.9736	7532.9760
4	32.0000	4.0000	21.7667	0.0318	714.6056	27223.1075	32.7586	216.6009	6801.8428
5	90.0000	1.5000	20.6179	0.0336	1568.2761	63578.1956	37.5648	106.3519	3163.4783
6	189.0000	1.0000	14.8180	0.0468	860.9027	15864.2587	17.6581	200.3530	4283.1274
7	205.0000	1.0000	15.7720	0.0439	1075.4284	19567.8955	17.4956	160.9377	3662.0026
8	112.0000	1.0000	18.1744	0.0381	760.4673	18532.6636	22.6741	220.2245	5774.3152
9	29.0000	4.0000	14.4163	0.0481	412.0297	8708.3885	20.2697	418.9686	8713.8699
10	173.0000	1.0000	22.3831	0.0310	775.7869	21464.5825	25.0475	210.0461	6782.8077
11	137.0000	4.0000	15.3034	0.0453	917.3115	18091.1495	18.8917	187.9652	4149.9133
12	98.0000	1.0000	13.5149	0.0513	847.1425	17611.4738	20.6069	210.6149	4106.5338
13	22.0000	8.0000	20.3625	0.0340	414.1120	13660.1733	29.2984	386.0652	11341.3766
14	72.0000	1.5000	13.3925	0.0518	861.6812	17876.4526	20.0641	202.0279	3903.4266
15	18.0000	2.0000	22.8789	0.0303	287.0492	15355.1412	35.3724	414.6511	13686.4874
16	352.0000	1.0000	16.7276	0.0414	1776.7740	28921.0944	15.6367	97.3199	2348.5975
17	71.0000	1.0000	16.0919	0.0431	425.4220	9436.7117	19.1524	365.3221	8481.1888
mean	133.412	2.029	17.529	0.040	860.840	22961.766	24.461	192.464	6077.968
SD	97.014	1.932	5.595	0.009	387.238	13934.800	7.505	102.557	3082.602

* Values were calculated using harmonic mean.

Table 7 A comparison of Pharmacokinetic parameter of Xanthohumol between Kinetica and WinNonlin software

Parameter	Unit	Low dose			Medium dose			High dose		
		Kinetica	WinNonlin	p-value	Kinetica	WinNonlin	p-value	Kinetica	WinNonlin	p-value
C_{max}	µg/L	45.167	45.313	0.1545	66.874	66.846	0.7563	133.285	133.412	0.1018
T_{max}	h	0.781	0.781	N/A	1.231	1.231	N/A	2.029	2.029	N/A
$t_{1/2}$	h	2.084	2.060	0.9804	10.339	10.516	0.4969	16.953	17.529	0.1256
k	h^{-1}	0.333	0.336	0.6478	0.067	0.066	0.5415	0.041	0.040	0.2666
AUC	(h)*(µg/L)	109.812	111.034	0.2238	323.026	322.653	0.8813	862.585	860.840	0.4937
AUMC	h^2*(µg/L)	1566.699	2395.283	0.2903	12126.749	17741.892	0.4534	22110.271	22961.766	0.1421
MRT	h	10.601	11.911	0.3035	26.443	31.252	0.4998	23.749	24.461	0.1336
Cl/F	L h^{-1}	156.789	147.665	0.8551	160.016	154.029	0.5057	193.813	192.464	0.5343
V_z/F	L	1909.321	1889.934	0.6208	4213.498	4398.289	0.5446	5831.857	6077.968	0.1052



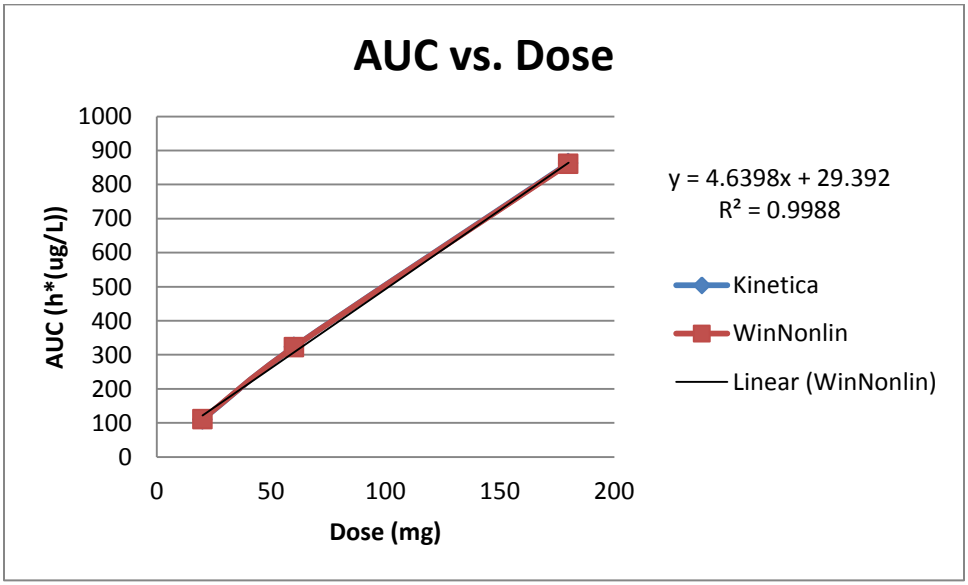


Figure 7 Plot of C_{max} versus dose and AUC versus dose demonstrating the linearity of pharmacokinetics for Xanthohumol.

ISOXANTHOHUMOL

There were no results presented from low dose (20 mg) subject group because the isoxanthohumol plasma concentrations were not detectable in all subjects in this group.

The mean plasma concentrations of isoxanthohumol versus time curves after oral administration of Xanthohumol are shown in Figures 8.1 and 9.1, for medium and high dose, respectively. The semilogarithmic plots of plasma concentration of isoxanthohumol vs. time after administration of Xanthohumol are shown in Figure 8.2 (medium dose) and Figure 9.2 (high dose).

From the curves, after administration the isoxanthohumol concentration increased rapidly in both groups and reach peak concentrations in 8 and 5 hours for medium and high dose, respectively and a second peak occurred around 24 hours. The second peak can be seen in the plot of mean plasma concentration of isoxanthohumol vs. time of the medium dose group. On the other hand, for the high dose (180mg) there appears to be no second peak showing in Figure 9. However, each individual subject showed a second isoxanthohumol peak. The concentrations declined after the second peak and all isoxanthohumol was eliminated in 4 days.

The PK parameters from Kinetica software from the two doses are listed in Tables 8 and 9. The PK values of subjects no.4 in medium dose (60 mg) and no.15 in high dose (180mg) were calculated by hand because the program could not fit these two subjects data.

The PK parameters from WinNonlin software for the three doses are listed in Tables 10 and 11. The PK values of subjects no. 4 in medium dose (60 mg) and no.15 in high dose (180mg) were calculated by hand because the program could not fit these data. The same result from the manual calculation performed for the Kinetica analysis was used in the WinNonlin analysis.

The C_{max} are around 9 and 31 ug/L and T_{max} are 7 and 5 hours for medium and high dose respectively. C_{max} is the highest drug concentration after administration whereas T_{max} is the time to reach C_{max}. In this case isoxanthohumol in high dose group was produced at a greater extent than the medium dose group and T_{max} in both groups of isoxanthohumol was later than xanthohumol. For the elimination phase, isoxanthohumol was excreted with an elimination rate constant (k) of 0.038 and 0.03451hr^{-1} in medium and high dose groups. Isoxanthohumol had a high volume distribution in both groups.

A comparison results between two programs shows almost all pharmacokinetic parameters in both groups are not different. Only AUC in medium dose reveals a different value between the two programs, p-value 0.04404, though 283.653 and 289.184 h*(µg/L) seem barely different.

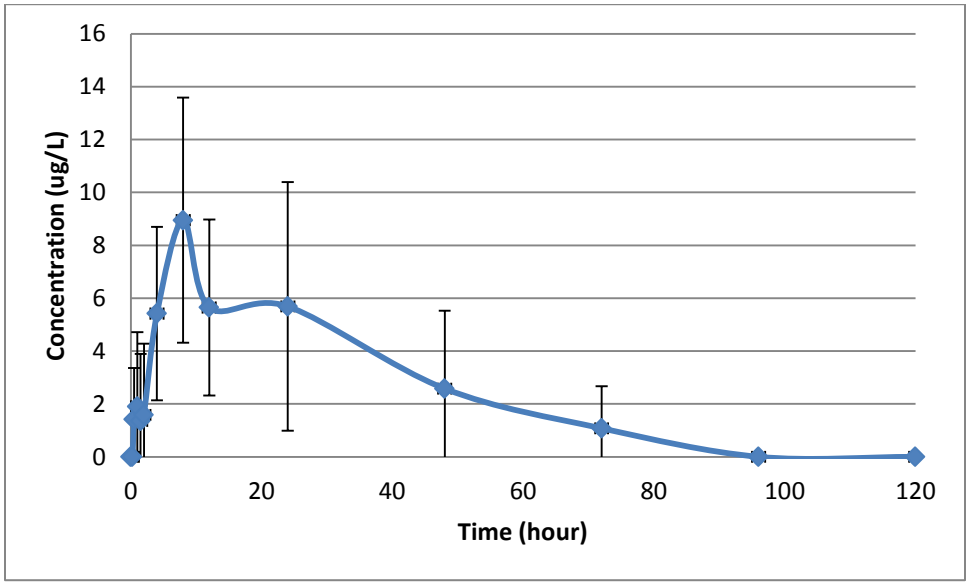


Figure 8.1 Plot of mean plasma concentrations of Isoxanthohumol vs. time after administration of Xanthohumol 60mg

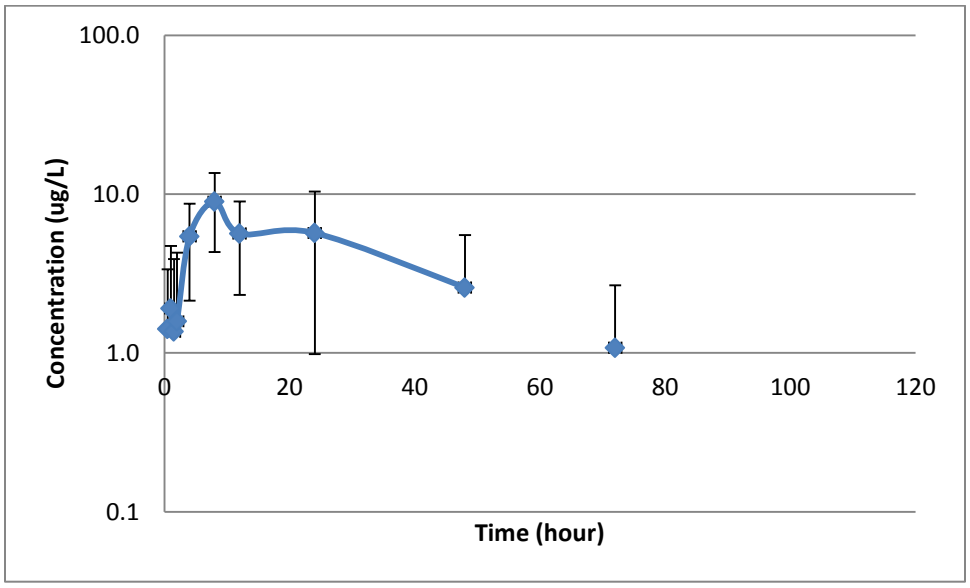


Figure 8.2 Semilogarithmic plot of mean plasma concentrations of Isoxanthohumol vs. time after administration of Xanthohumol 60mg

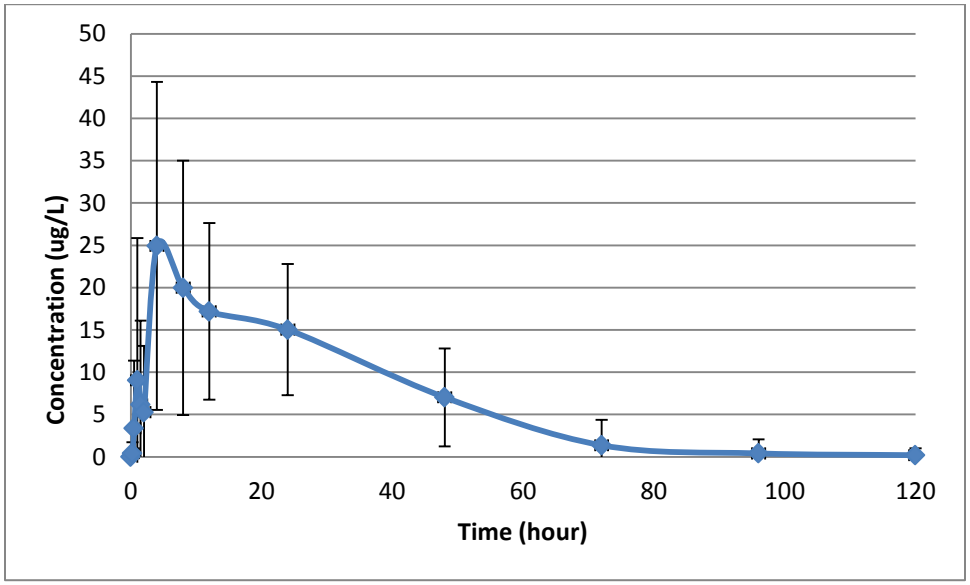


Figure 9.1 Plot of mean plasma concentrations of Isoxanthohumol vs. time after administration of Xanthohumol 180mg

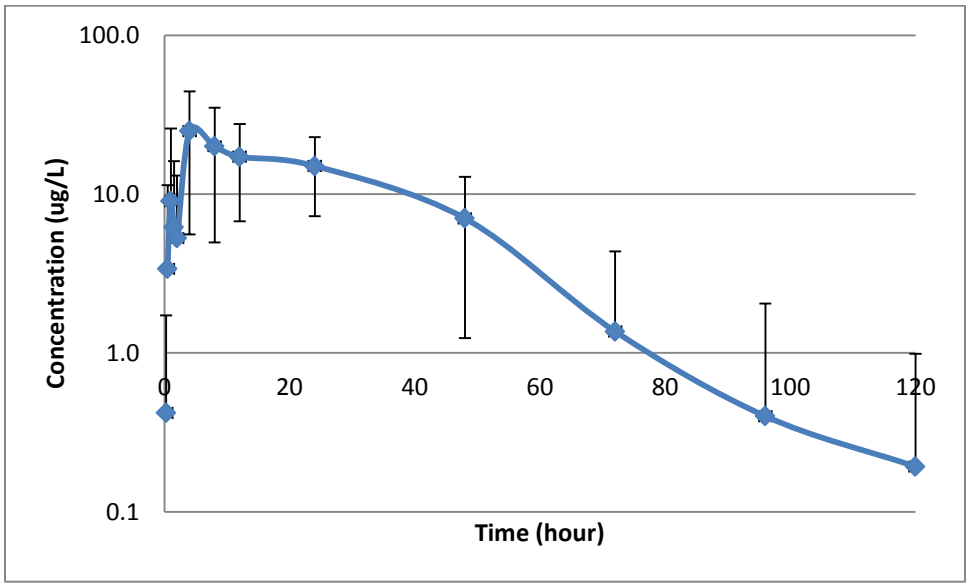


Figure 9.2 Semilogarithmic plot of mean plasma concentrations vs. time after administration of Isoxanthohumol 180mg

Table 8 The Pharmacokinetic parameters from Kinetica software of Isoxanthohumol for 60mg Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	4.9383	2.0000	49.6263	0.0140	151.1251	27194.4816	84.6187	186.6967	13366.6767
2	10.2129	8.0000	73.7667	0.0094	517.1726	111628.2284	112.1712	60.2918	6416.4259
3	8.7615	8.0000	10.8595	0.0638	127.5865	3057.4082	22.1036	433.7711	6795.8762
4	5.1153	8.0000	19.2714	0.0360	55.7274	496.3800	8.9139	1077.4701	9604.4717
5	15.3813	24.0000	20.5912	0.0337	731.5010	31618.3126	40.2459	76.3721	2268.7771
6	7.0623	8.0000	8.3606	0.0829	102.0483	1636.0866	16.0161	587.3548	7084.5818
7	14.1069	8.0000	16.5450	0.0419	479.3180	16780.8669	33.6401	120.2802	2871.0089
8	10.3368	8.0000	14.8627	0.0466	360.6286	11527.5424	31.2186	162.4904	3484.1726
9	5.5224	8.0000	16.6691	0.0416	149.6869	4441.7655	27.5430	372.0543	8947.3097
10	8.0004	8.0000	20.5902	0.0337	226.6931	8735.8343	36.2084	248.6886	7387.3913
11	4.3471	0.5000	48.6574	0.0142	100.3693	19816.7007	74.0660	224.2534	15742.0781
12	18.6381	8.0000	21.9027	0.0316	534.1986	21192.2302	36.3347	102.8717	3250.6418
13	10.9386	4.0000	12.5742	0.0551	151.4380	3518.5712	20.6036	351.3409	6373.6049
mean	9.489	7.885	17.860	0.039	283.653	20126.493	41.822	166.293	7199.463
SD	4.413	5.508	19.352	0.021	216.256	29313.142	30.077	278.488	4018.708

* Values were calculated using harmonic mean

Table 9 The Pharmacokinetic parameters from Kinetica software of Isoxanthohumol for 180mg Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	43.5420	4.0000	26.1202	0.0265	1140.8084	55143.8537	39.0985	127.6248	4809.3400
2	41.9490	4.0000	12.2698	0.0565	1167.7308	28981.3135	24.7562	153.7584	2721.7638
3	27.5943	4.0000	40.9917	0.0169	920.8177	85749.9107	60.2205	126.4105	7475.7350
4	25.3464	8.0000	18.8821	0.0367	763.6560	25987.7684	32.8909	227.8134	6205.8868
5	28.5324	4.0000	24.9381	0.0278	1531.5066	95996.0724	58.2058	109.1403	3926.6527
6	66.1980	8.0000	10.6263	0.0652	1174.2276	24471.9415	20.7859	152.8878	2343.8380
7	61.2420	4.0000	18.7814	0.0369	830.5136	25720.8332	28.2355	197.5982	5354.0823
8	27.6828	4.0000	22.3562	0.0310	973.7142	35489.1080	33.9475	172.1811	5553.3987
9	9.9120	4.0000	39.0010	0.0178	267.8141	23695.6910	57.9573	440.2621	24772.0333
10	25.3464	4.0000	35.5802	0.0195	530.1475	41006.7627	53.4262	234.5154	12038.0105
11	23.4525	4.0000	26.3339	0.0263	487.1119	25443.0146	41.3394	292.4608	11111.1011
12	21.2931	12.0000	22.0184	0.0315	578.5244	20646.8488	33.4439	291.5649	9261.7867
13	18.7797	8.0000	7.5617	0.0917	275.9142	4649.4765	17.1659	664.5605	7249.8260
14	11.0094	8.0000	23.5104	0.0295	345.0796	15638.8025	37.7979	435.0479	14756.1026
15	4.5489	4.0000	247.5000	0.0028	127.5159	2178.5000	17.2985	1429.2974	24724.6572
16	68.8530	1.0000	20.5498	0.0337	1280.3460	40618.4799	28.4115	125.9049	3732.7071
17	26.6208	4.0000	23.6286	0.0293	538.2184	20868.6927	35.4728	305.9654	10429.9930
mean	31.288	5.235	20.329	0.034	760.803	33663.945	36.497	206.537	9203.936
SD	19.070	2.635	55.119	0.021	409.538	25064.754	13.927	320.490	6788.677

* Values were calculated using harmonic mean

Table 10 The Pharmacokinetic parameters from WinNonlin software of Isoxanthohumol for 60mg Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	5.0000	2.0000	45.5519	0.0152	152.6814	25153.9989	80.1507	191.1841	12564.1280
2	10.0000	1.0000	70.7890	0.0098	524.2936	104823.9404	107.5197	61.5430	6285.1950
3	9.0000	8.0000	11.0653	0.0626	128.2035	3146.6316	22.4607	428.2804	6837.0114
4	5.0000	8.0000	19.2714	0.0360	55.6609	496.3800	8.9139	1077.4701	9604.4717
5	15.0000	8.0000	20.6725	0.0335	727.0816	31344.1890	40.1563	76.8685	2292.5321
6	7.0000	8.0000	9.4230	0.0736	112.0680	2016.0526	17.4918	520.5752	7077.0000
7	14.0000	8.0000	18.5689	0.0373	478.3849	18272.7806	35.9726	118.1187	3164.3189
8	10.0000	8.0000	15.1423	0.0458	374.2251	12386.7583	32.2512	156.2212	3412.7675
9	6.0000	8.0000	16.5378	0.0419	157.3674	4650.3355	27.4800	354.5545	8459.3174
10	8.0000	8.0000	26.4162	0.0262	239.6714	10946.4125	41.1857	225.7490	8603.4115
11	4.0000	0.5000	129.3696	0.0054	99.9843	118745.2406	190.3252	96.1682	17948.9172
12	19.0000	8.0000	23.8593	0.0291	561.4793	24939.3401	39.6653	95.4283	3284.8059
13	11.0000	4.0000	11.7921	0.0588	148.2963	3170.9482	19.4139	367.3465	6249.4521
mean	9.462	6.115	18.964*	0.037	289.184	27699.462	50.999	152.243*	7367.948
SD	4.427	3.042	33.685	0.020	217.969	38734.463	49.695	279.423	4332.171

* Values were calculated using harmonic mean

Table 11 The Pharmacokinetic parameters from WinNonlin software of Isoxanthohumol for 180mg Xanthohumol

Sample	Parameter								
	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	k (h^{-1})	AUC ((h)*(μg/L))	AUMC (h^2*(μg/L))	MRT (h)	Cl/F (L h^{-1})	V_z/F (L)
1	44.0000	4.0000	25.2271	0.0275	1122.7906	51502.1982	37.6825	131.7001	4793.2343
2	42.0000	4.0000	11.5025	0.0603	1191.9654	30620.4502	25.5904	150.4312	2496.3504
3	28.0000	4.0000	42.4554	0.0163	927.6787	91770.4494	62.4531	122.4964	7502.9264
4	25.0000	8.0000	17.7279	0.0391	756.9900	26160.2266	33.3616	229.5503	5870.9775
5	29.0000	4.0000	23.3639	0.0297	1523.8481	92721.5961	57.0605	110.7713	3733.7646
6	66.0000	8.0000	10.6180	0.0653	1162.1750	24171.9811	20.7397	154.4410	2365.8081
7	61.0000	4.0000	16.8136	0.0412	825.0212	23565.6857	26.5887	203.0901	4926.3486
8	28.0000	4.0000	23.4869	0.0295	986.8903	38282.1322	35.6302	167.5310	5676.7036
9	10.0000	4.0000	35.0753	0.0198	264.6052	19897.8531	52.3058	473.1684	23943.7521
10	25.0000	4.0000	54.9499	0.0126	527.7348	70757.8768	75.9702	193.2594	15320.8248
11	23.0000	4.0000	27.1492	0.0255	478.7782	26173.7047	42.5853	292.8646	11470.9385
12	21.0000	12.0000	19.7554	0.0351	584.2950	20076.7863	32.5237	291.5939	8310.7025
13	19.0000	8.0000	7.7107	0.0899	287.5945	4951.7235	17.4840	635.5593	7070.0378
14	11.0000	8.0000	22.0074	0.0315	340.8815	14491.8774	36.2178	449.8522	14282.7911
15	5.0000	4.0000	247.5000	0.0028	126.1609	2178.5000	17.2985	1429.2974	24724.6572
16	69.0000	1.0000	18.7789	0.0369	1264.6877	37007.0195	26.7117	129.9240	3519.9230
17	27.0000	4.0000	23.3352	0.0297	518.8672	19048.0243	33.8813	320.1717	10778.7654
mean	31.353	5.235	19.883*	0.035	758.292	34904.593	37.299	205.801*	9222.853
SD	19.026	2.635	55.505	0.021	408.500	26983.156	16.311	320.503	6857.973

* Values were calculated using harmonic mean

Table 12 A comparison of Pharmacokinetic parameter of Isoxanthohumol between Kinetica and WinNonlin software

Parameter	Unit	Medium dose			High dose		
		Kinetica	WinNonlin	p-value	Kinetica	WinNonlin	p-value
C_{max}	µg/L	9.489	9.462	0.7153	31.288	31.353	0.4234
T_{max}	h	7.885	6.115	0.1991	5.235	5.235	N/A
$t_{1/2}$	h	17.860	18.964	0.3185	20.329	19.883	0.7490
k	h^{-1}	0.039	0.037	0.0690	0.034	0.035	0.2618
AUC	(h)*(µg/L)	283.653	289.184	0.0440	760.803	758.292	0.3902
AUMC	h^2*(µg/L)	20126.493	27699.462	0.3414	33663.945	34904.593	0.5200
MRT	h	41.822	50.999	0.3257	36.497	37.299	0.5822
Cl/F	L h^{-1}	166.293	152.243	0.1179	206.537	205.801	0.9848
V_z/F	L	7199.463	7367.948	0.4415	9203.936	9222.853	0.9329

DISCUSSION

XANTHOHUMOL (XN) AND ISOXANTHOHUMOL (IX)

Xanthohumol is a natural substance from the female flowers of the hops plant (Humulus lupulus L.). In nature, there is only small amount of xanthohumol. In order to improve the benefit of xanthohumol, many companies try to extract, develop and produce a formulation to launch as a nutritional supplement on the market. The one problem of xanthohumol is a specific chemical property. It is nearly insoluble in water.

This study used a microemulsion formulation of xanthohumol. Microemulsion formulations help drug delivery systems improve bioavailability, especially for an insoluble drugs. Microemulsions are used in many drug formulations such as parenteral, oral or transdermal drug delivery systems in order to improve drug dissolution and bioavailability of drug. The main components in a microemulsion are surfactant, oil phase and aqueous phase. For this study, XN in powder form was dissolved into an isotropic mixture of oleic acid, Tween 80 and propylene glycol to promote maximum absorption. Capsules were filled with this emulsion with a dose (20, 60 and 180 mg) of xanthohumol. Each subject took one capsule of the respective dose during the study.

From the plasma concentration versus time curves of xanthohumol, the results show two high concentration peaks in all of three dose groups. In accordance with the formulation, xanthohumol was dissolved in microemulsion. When xanthohumol enters the stomach, the capsule shell dissolves and releases the microemulsion. Because of the high acidic fluid content in the stomach of humans, a portion of the xanthohumol precipitates and separates from microemulsion. The dissolved xanthohumol is readily absorbed into the blood circulation and produces the first drug peak at about 2 hours. After that the precipitated portion of xanthohumol slowly dissolves and produces a second drug peak a couple hours later. The two peaks are shown in plasma versus time curves of xanthohumol but also in isoxanthohumol plasma concentration time curves.

The delay in isoxanthohumol appearance in the plasma confirms that the isoxanthohumol is one of the metabolites of xanthohumol.

The T_{max} pharmacokinetic parameter values for both xanthohumol and isoxanthohumol are related. Isoxanthohumol appearance in plasma is predicated upon the biotransformation process in the human body of xanthohumol to isoxanthohumol. Figures 10 and 11 show an overlay the plasma concentration time curves for xanthohumol and isoxanthohumol from the medium and high dose data. In both groups, T_{max} of xanthohumol is always less than isoxanthohumol. When the concentration of xanthohumol decreases after the first highest peak, the concentration of isoxanthohumol is climbing to the C_{max} and declines a couple hours later.

The elimination rate constant (k) and half-life for both xanthohumol and isoxanthohumol are also related. For the xanthohumol group, the half-lives range between 2-16 hours. Isoxanthohumol stays in human body longer than xanthohumol, $t_{1/2} \approx$ 17-20 hours. In addition, the half-life value for xanthohumol increases as the dose increases.

C_{max} and AUC were determined for both xanthohumol and isoxanthohumol. The dose administered of xanthohumol is related to both C_{max} and AUC in direct fashion indicating linear pharmacokinetics. This means that xanthohumol pharmacokinetic parameters are not time or dose dependent. There are no other factors such as absorption, protein binding, first-pass metabolism and excretion involved with this drug's pharmacokinetics. Unfortunately there are not enough data to test whether isoxanthohumol follows linear pharmacokinetics due to the lack of data for 20 mg oral dose subjects.

For the volume of distribution at steady state (V_{ss}), only Kinetica's software can estimate and report volume of distribution. In the WinNonlin program, the study used the model 200 (non-compartmental analysis, extravascular) and only this model in the

program is the volume of distribution not computed. However in the guidelines of WinNonlin is shown the equation for manual calculation for volume of distribution:

$$V_{ss}/F = MRT_{inf} * Cl$$

*MRTinf and Cl come from the estimated fitted values of the program.

The comparison of V_{ss} values between these two programs are shown in Tables 13 and 14 for xanthohumol and isoxanthohumol. All results indicate that both programs estimate similar results for this pharmacokinetic parameter, p-value > 0.05.

The comparison of results of pharmacokinetic parameters provided by Kinetica and WinNonlin show that both programs give similar values for both xanthohumol and isoxanthohumol. The p-values of all parameters are above 0.05.

8-PRENYLNARINGENIN (8PN)

There are no results of this metabolite because the plasma drug concentrations were undetectable in all subjects.

In nature there are both xanthohumol and isoxanthohumol in hops. Hops (*Humulus lupulus* L.) have been used in brewing beer for bitterness and as a flavoring agent. Almost 90% of the prenylated flavonoid is xanthohumol and the rest is isoxanthohumol. In vitro studies show that xanthohumol can be converted to isoxanthohumol through cyclization in acidic conditions (0.05%HCl) similar to gastric juice in human body.[16] However, isoxanthohumol has only moderate estrogenic activity therefore some studies have changed their focus to another metabolite called 8-prenylnaringenin (8PN). Results in simulated human intestinal microbial ecosystem (SHIME) indicate that isoxanthohumol passes unaltered through the stomach and small intestine. The conversion from isoxanthohumol to 8PN occurs only in the distal colon where up to 80% conversion is seen.[20] Moreover interindividual differences in the

intestinal microbial community are also important in this conversion.[18] 8PN can be transformed only from isoxanthohumol.

In this study the volunteers were administered only xanthohumol in capsules containing 20, 60 and 180mg doses. Because the amount of 8PN depends on many factors and the site of transformation occurs only in the colon parts, taking plasma samples may not be the good way to detect this metabolite. It was impossible to determine an amount of this metabolite in high enough levels since 8PN can only be obtained from demethylation of isoxanthohumol.

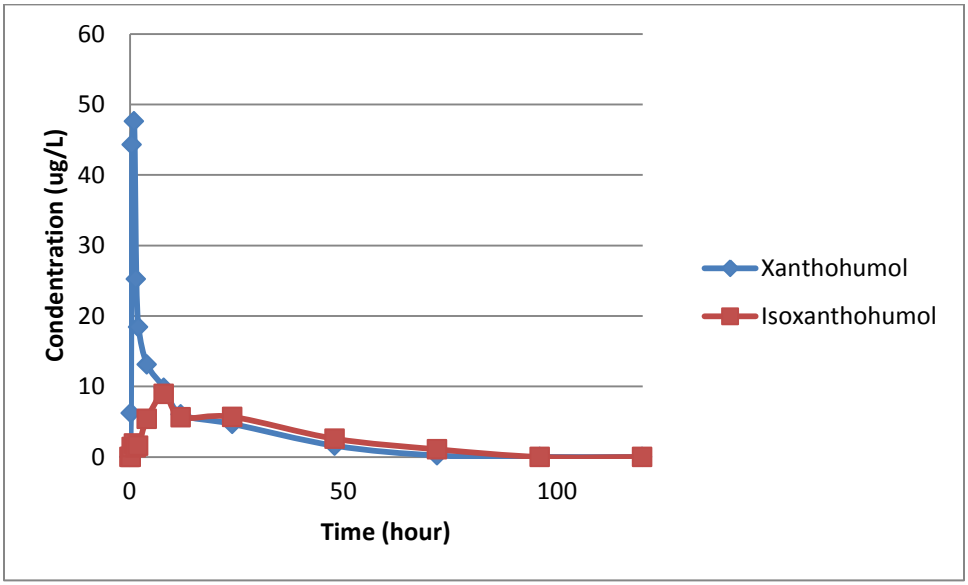


Figure 10 Plasma drug concentrations vs. time curve in *medium* dose group between Xanthohumol and Isoxanthohumol

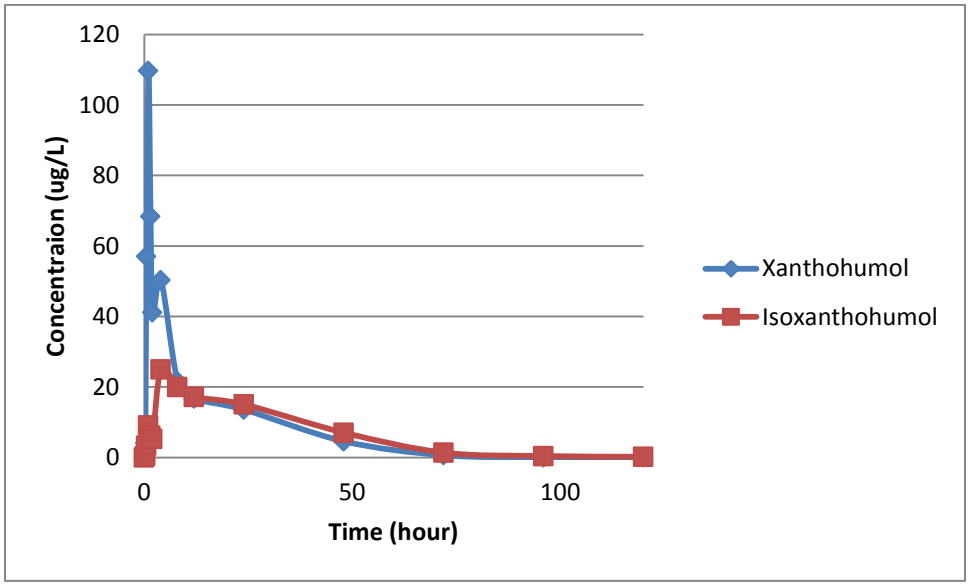


Figure 11 Plasma drug concentrations vs. time curve in *high dose* group between Xanthohumol and Isoxanthohumol

Table 13 A comparison of Volume of distribution (V_{ss}) of Xanthohumol between Kinetica and WinNonlin software

	Low dose		Medium dose		High dose	
	Kinetica	WinNonlin	Kinetica	WinNonlin	Kinetica	WinNonlin
1	3090.7237	2993.8350	7464.1064	6446.6985	4775.8961	4560.7306
2	1316.7425	2023.4726	8941.7567	11927.5018	3293.0361	3341.3088
3	2360.9582	2299.4306	5745.1693	5688.8670	5293.5376	5555.5122
4	1967.9204	1967.9204	3586.2799	3403.8668	6808.9791	7095.5422
5	821.9467	834.3835	2554.6751	2602.7738	3910.0605	3995.0879
6	482.8629	482.6793	3271.8150	3297.6056	3313.3575	3537.8533
7	729.5674	777.7292	4734.4825	4599.7963	2789.0334	2815.7016
8	508.0522	510.0559	1827.5571	1819.0978	4937.6548	4993.3923
9	2558.0761	2659.3131	3371.0336	3394.5466	8852.5192	8492.3678
10	2611.7059	2550.2649	2456.5993	2459.3396	4647.1652	5261.1297
11	2774.4561	2417.7074	3411.6490	3489.2316	3609.6999	3550.9822
12	2772.8394	2360.3442	2800.7161	2611.6185	4512.4287	4340.1202
13	6315.7895	6315.7895	3370.7688	3499.1503	10750.7963	11311.0927
14	3027.7168	4436.1532			4140.3128	4053.5080
15	3223.9774	3216.4777			14114.2401	14667.2046
16	2990.4441	3063.4714			1528.5700	1521.7621
17	1348.5892	1325.0749			6905.4716	6996.7950
18	1802.4109	1815.4065				
mean	1960.0796*	1956.0979*	4118.2007	4249.2380	5540.1623	5652.3583
SD	970.1181	915.2454	2087.5103	2645.3252	3162.1406	3291.9803
p-value	0.4387		0.6114		0.1136	

* These mean values are excepted subject no.13 and 14.

Table 14 A comparison of Volume of distribution (V_{ss}) of Isoxanthohumol between Kinetica and WinNonlin software

	Medium dose		High dose	
	Kinetica	WinNonlin	Kinetica	WinNonlin
1	15798.0258	15323.5394	4989.9333	4962.7890
2	6763.0033	6617.0849	3806.4793	3849.5946
3	9587.8984	9619.4776	7612.5045	7650.2799
4	9604.4717	9604.4717	7492.9904	7658.1653
5	3073.6677	3086.7545	6352.5992	6320.6658
6	9407.1077	9105.7973	3177.9087	3203.0600
7	4046.2393	4249.0367	5579.2832	5399.9017
8	5072.7220	5038.3212	5845.1241	5969.1630
9	10247.4810	9743.1577	25516.4060	24749.4517
10	9004.6132	9297.6306	12529.2698	14681.9553
11	16609.5565	18303.2319	12090.1450	12471.7269
12	3737.8130	3785.1921	9751.0582	9483.7125
13	7238.8999	7131.6282	11407.7657	11112.1188
14			16443.9118	16292.6570
15			24724.6572	24724.6572
16			3577.1441	3470.4909
17			10853.4357	10847.8334
mean	8476.2692	8531.1788	10102.9774	10167.5425
SD	4221.6365	4422.2685	6735.1927	6697.5733
p-value	0.7227		0.6579	

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CHAPTER 3

LIPOIC ACID

Chanida Karnpracha, J. Mark Christensen

INTRODUCTION

Lipoic acid (LA) is also known as alpha lipoic acid or thioctic acid. There are two sulfur atoms in the molecule linked together with a disulfide bond. The carbon atom (C6) is a chiral atom therefore it has two enantiomers, (*R*)-(+)-lipoic acid (RLA) and (*S*)-(-)-lipoic acid (SLA) and can exist as a racemic mixture (*R*/*S*)-lipoic acid (R/S-LA).

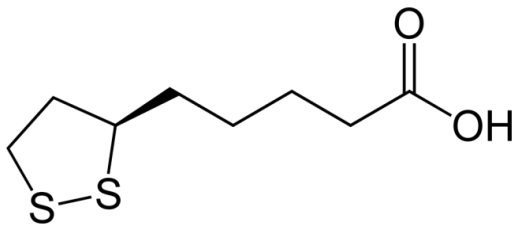

Figure 12 A structure of lipoic acid.

Alpha lipoic acid can be found in nature and obtained naturally in our diet. Red meat is a good source of lipoic acid. R-form of lipoic acid occurs naturally in food but the synthetic product is racemic mixture, combining R- and S- forms. In mammalian cells, lipoic acid is produced by de novo biosynthesis. Cysteine is the source of sulfur and octanoate is an intermediate fatty acid.[1] Alpha lipoic acid plays a crucial role in metabolic systems in all organisms from bacteria to humans. Lipoic acid works as a co-factor of pyruvate dehydrogenase in TCA cycle and α-ketoglutarate dehydrogenase complexes. After being absorbed lipoic acid is converted to its reduced form, dihydrolipoic acid (DHLA) by NADH or NADPH. Both lipoic acid and its reduced form are anti-oxidants. They act as free radical scavengers. [2]

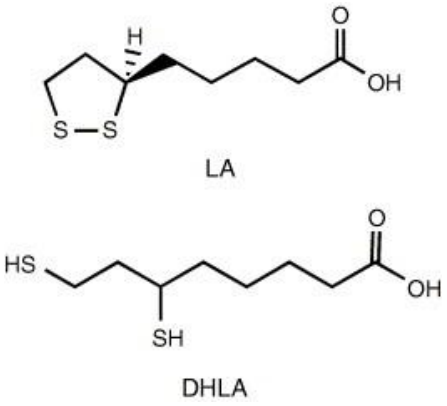

Figure 13 Structure of lipoic acid (LA) and dihydrolipoic acid (DHLA) [3]

The initial activity known for lipoic acid was related to diabetes. Because protein glycation may play an important role in diabetic complications, alpha lipoic acid prevents the glycation of bovine serum albumin (BSA) near the glycation sites of BSA.[4] Lipoic acid supplementation improves nerve blood flow (NBF) by reducing the effects of oxidative stress. Decreasing blood levels of reduced glutathione is an indicator for oxidative stress, suggesting, lipoic acid may be used as a drug for human diabetic neuropathy.[5] Moreover, a concurrent study in diabetic patients showed that alpha lipoic acid can reduce symptoms of diabetic peripheral neuropathy. A diabetic neuropathy is a type of nerve damage in diabetic patients. The most often damages are occurred in leg and foot. Lipoic acid 600 mg/day over 3 weeks were given without any side effects in this study.[6] Lipoic acid can also lower blood glucose by enhancing glucose uptake and GLUT1 and GLUT4 translocation.[7]

Lipoic acid acts as an antioxidant. In HIV patients the transcription of DNA is activated by binding between the long terminal repeat region of DNA and nuclear factor kappa B (NF-kappa B). A reactive oxygen species (ROS) is involved in this the activation of NF-kappa B. Alpha lipoic acid inhibits this process by removing all reactive oxygen species.[8] In Alzheimer's disease, alpha lipoic acid and dihydrolipoic acid

inhibit the formation, extension and destabilization of β-amyloid fibrils (fAβ) in the CNS therefore be an important site for development of therapeutic agents for the disease.[3] Lipoic acid 1200 mg was studied in patient with multiple sclerosis (MS) and was shown that taking this high dose once daily can achieve therapeutic serum levels in MS subjects.[9]

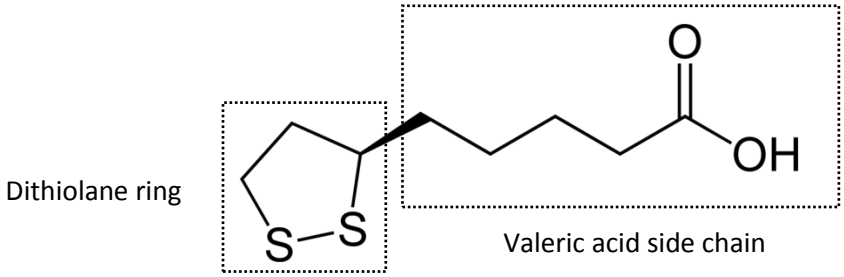

Figure 14 Dithiolane ring and valeric acid side chain of lipoic acid.

Lipoic acid is soluble in ethanol and water (as a sodium salt). A partition coefficient of lipoic acid is approximately 4/1 (o/w), meaning that lipoic acid is soluble in both aqueous and non-aqueous media.[34] Lipoic acid was mostly absorbed over the whole intestinal tract. From in-situ ligated segments of the gastrointestinal tract of the rat, lipoic acid was absorbed poorly in stomach, about 35%, by passive diffusion of the unionized form of lipoic acid (pK_a3.5) and by mediated diffusion in the intestinal tract (duodenum, jejunum, ileum and colon).[10] However, a study in Caco-2 cell monolayers showed that lipoic acid was absorbed by mediated transport and was affected by the pH of the environment. Monocarboxylic acids such as benzoic acid and medium-chain fatty acid significantly prevent the transportation of lipoic acid therefore a monocarboxylic acid transporter (MCT) should be involved in transportation in the intestine.[11] Na^+-dependent multivitamin transporter facilitates transport of lipoic acid from blood or plasma into tissues.[12,13] The sodium salt of the R-enantiomer is more water soluble and has higher absorption than the unionized R- and racemic forms. The salt of the R-enantiomer changes to the free acid in the acid condition of the stomach and slows down absorption in the alkaline environment.[14]

The presence of concurrent administration of food affects absorption of both enantiomers of lipoic acid. Lipoic acid given orally to healthy volunteers revealed that AUC and C_{max} in the fasting group were higher than fed group. Moreover T_{max} of fasting group was also lower than the fed group.[15] A pharmacokinetic study in dogs indicated that the inclusion of racemic of lipoic acid in dog food decreased C_{max} and delayed T_{max} compared to oral administration with a capsule.[16] Lipoic acid was given to elderly and younger subjects. The elderly volunteers exhibited a higher C_{max} and AUC of the R-isoform than the racemic mixture while the C_{max} and AUC of racemic form were greater than R-form in younger age group. The bioavailability was more variable in elder subjects compared to the younger age group suggesting an age dependent difference in pharmacokinetics between the two groups.[17]

The biotransformation investigation in rat and an in vitro study with rat liver system determined that alpha lipoic acid was metabolized by β-oxidation in the valeric acid side chain and other reactions on dithiolane ring. Ion-exchange and paper chromatography of urine of rat obtained 24 hours after oral administration revealed that most metabolites were water-soluble.[18] The metabolic pathways of lipoic acid in several animal species are different. In human the metabolic pathway closely resembles the observed pathway in mice and rat. The major process is β-oxidation, producing 3-keto lipoic acid, the initial metabolite. Subsequently S-methylation is involved. Other mechanisms such as oxidation of methy sulfides, forming methy sulfoxide occurred in all species (mice, rat, dog and human).[19]

Of the administered dose only 12.4% of lipoic acid and its metabolites were recovered after 24 hours by urinary excretion.[20] Severe kidney damage or end-stage renal disease did not influence the pharmacokinetics of lipoic acid. There was no need to adjust the dose of alpha lipoic acid in patients with renal dysfunction.[21] However the animal models show that more than 80% of the administered radioactive-labeled dose is recovered in urine.

The concentration of R- and S- form climbed rapidly, from 0.5-1 hour in healthy, fasting volunteers. The terminal plasma half-life was short (0.47-0.64 hour) for both enantiomers and did not show any dose-dependency. The clearance of the S-enantiomer was higher than the R-enantiomer but also did not show any dose-dependency. Dose-proportional (50-600 mg) pharmacokinetics (AUC versus dose) could be demonstrated for both enantiomers on an intra-individual basis as well as for the group geometric means.[22]

The R-form of lipoic acid had a significantly higher bioavailability than the S-form. The pharmacokinetics study in healthy volunteers revealed that in all formulations (iv, solution and tablets) of lipoic acid, the AUC and C_{max} values of the R-form were higher than the S-form. However the bioavailabilities of both enantiomers were low, from 16-38% in all formulations. The reasons were lipoic acid is excreted mainly in liver at a high rate and had incomplete absorption. [23]

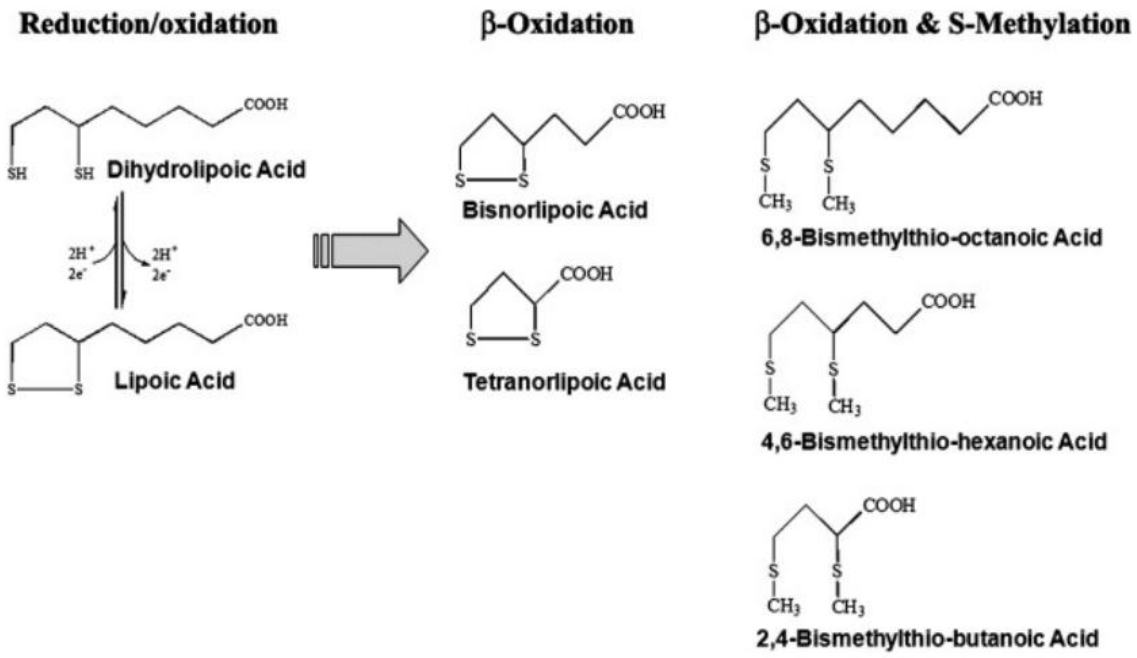

Figure 15 Lipoic acid and its reduced form, dihydrolipoic acid, along with the 5 most common metabolites.[24]

MATERIALS AND METHODS

PHARMACOKINETICS SOFTWARE

There were two pharmacokinetic software programs used in this study, Kinetica version 5.0 (Thermo Scientific) and WinNonlin version 5.3 (Pharsight Cormporation).

Kinetica is a pharmacokinetic-pharmacodynamic (PK/PD) template and method-driven system developed by Thermo Scientific. There are both default validated methods, more than 50 different methods and customized methods. The validated methods can be saved as the template and used for other analysis to provide a consistency during analyses and for the analysts. The templates and methods in Kinetica are made in only one interface including a series of panes, spreadsheets, graphical and web views. Moreover in bioequivalence studies, Kinetica provides the statistics package that meets the requirements of the FDA, EMEA and MHW regulatory guidelines. Kinetica offers fast, high-throughput data analysis for discovery, preclinical, clinical, drug metabolism and drug delivery settings.

Kinetica is installed with nine subdirectories:

- Absorption Kinetics
- Compartmental Fitting
- Convolution/Deconvolution
- Enzyme Kinematics
- In Vivo/In Vitro Correlation
- Non-Compartmental Analyses
- Population Pharmacokinetics
- Protein Binding
- Urine Pharmacokinetics

WinNonlin is distributed by Pharsight Corporation. There are compartmental and noncompartmental models in order to analyze the raw data from pharmacokinetic and pharmacology studies. In addition, the user can also develop templates and save them as user models for use next time. WinNonlin provides a large library of pharmacokinetic, pharmacodynamic, noncompartmental, PK/PD link, and indirect response models.

WinNonlin is a tool for non-linear modeling. It can be fit differential equations, algebraic equations or the combination of both in pharmacokinetic modeling. WinNonlin will not provide the results that show the correctness but it shows how good the model fits the data. Least squares is used in this program to fit concentrations versus

time data. Moreover, within WinNonlin a descriptive statistic package provides mean, mode, median, variance and standard deviation.

METHODS

Noncompartmental analysis was performed on the lipoic acid concentration versus time data using both Kinetica and WinNonlin software. Noncompartmental analysis is performed when compartmental analysis can not specify whether a one, two or three compartment model can fit the data. Noncompartmental analysis uses the area under the curve (AUC) to estimate the exposure of drug.

AUC is calculated by using the trapezoidal rules.

$$AUC = \frac{1}{2}(C_1 + C_2)(t_2 - t_1)$$

* C is the blood concentration and t is the sampling time point.

In Kinetica software, there are three types of calculation:

- Linear: computes all values in a linear manner.
- Log linear: computes all values prior to C_{max} in a linear manner and transforms all values after C_{max} to log

in its computation.

- Mixed log linear: the computation method where all ascending phases are computed in a linear manner and all descending phases are computed based on their log values.

For WinNonlin, there are four calculation methods of AUC as following:

- Linear/log trapezoidal: log-trapezoidal rule, using linear interpolation between log-transformed data through C_{last}.
- Linear trapezoidal (linear interpolate): linear trapezoidal rule, using linear interpolation between untransformed data through C_{last}
- Linear up/log down: linear trapezoidal rule up to C_{max}, log-trapezoidal rule after C_{max}; uses linear interpolation between untransformed data up to C_{max}, log-transformed data from C_{max} through C_{last}.
- Linear trapezoidal (linear/log interpolation): trapezoidal rule with linear interpolation between data points up to C_{max}, log-linear interpolation between data points from C_{max}, extrapolated to Tinf.

In this study the mixed log linear for Kinetica and linear up/log down for WinNonlin was used to fit the data. These two options are more accurate than other linear or log trapezoidal combination or used alone. Linear trapezoidal method is the best approximation in the absorption phase, increasing part, while logarithmic trapezoidal method best fits the elimination phase, decreasing area. Log trapezoidal AUC is calculated by this following equation:

$$AUC = \frac{C_1 - C_2}{\ln(C_1) - \ln(C_2)}(t_2 - t_1)$$

In WinNonlin, we used the uniform weighing of the data being fitted. However there was no choice in Kinetica software where uniform weighting of the data in only available.

PHARMACOKINETIC PARAMETER

The elimination constant (k) was calculated by the terminal slope during the elimination phase in each subject using the regression data analysis in Microsoft office excel 2007. AUC and AUMC are calculated by the trapezoidal rule. MRT (mean residence time) was determined by using the following equation:

$$MRT = AUMC/AUC$$

The other parameters were calculated by using these following equations:

$$t_{1/2} = 0.693/k$$

$$Cl/F = dose/AUC$$

$$V_{ss}/F = Cl*MRT$$

* F is bioavailability of drug.

MODEL DRUG

Lipoic acid oral doses (R-form and racemic form): 500 mg

Lipoic acid is now available on the market as an antioxidant supplement. However the supplement dose or therapeutic dose has not been defined. The contents of alpha lipoic acid in supplemental product vary from 50, 100, 200, 300, 400, 500 and 600mg. The data used in this study is from 500 mg R-lipoic acid and 500 mg racemic lipoic acid administered orally to healthy volunteers.

ANALYZING COMPONENTS

Two forms of lipoic acid: racemic and r-form

SUBJECTS

A study was conducted in healthy volunteers (n=19), a crossover study.

STATISTICS

The comparison between the pharmacokinetic results obtained from Kinetica and WinNonlin programs was performed using a paired t-test in R program. A probability level of 0.05 or less was considered to indicate statistical significance.

RESULTS

RACEMIC AND R FORM

The mean plasma concentrations versus time curves after oral administration of R-form and racemic form of lipoic acid are shown in Figures 16.1 and 17.1, respectively. The semilogarithmic plots of plasma concentrations vs. time after oral administration of R-form and racemic forms of lipoic are shown in Figures 16.2 and 17.2, respectively.

Lipoic acid is absorbed rapidly from both dosage forms, 40 minutes for racemic form and 30 minutes for R-form. The C_{max} and AUC values of racemic lipoic acid are comparable to the R-enantiomer, around 2400 ng/mL for Cmax and 115000 min*(ng/mL) for AUC. The elimination rate constant (k) and half-life ($t_{1/2}$) showed considerable differences between two programs. However lipoic acid is eliminated rapidly. The half-life values for both R- and racemic lipoic acid are short, within one hour. The total clearance is around 4600 ml/min.

The PK parameters obtained from Kinetica software are listed in Tables 15 and 16. The PK values of subject no. 11 in R-form were calculated by hand because the program could not fit this subject's data.

The PK parameters obtained from WinNonlin software are listed in Tables 17 and 18. The PK values of subject no. 11 in R-form were calculated by hand because the program could not fit this subject's data. The results of same manual calculation used in the Kinetica analysis were used for this subject in the WinNonlin data analysis.

For the volume of distribution at steady state (V_{ss}), only the Kinetica software estimates and reports a value. In the WinNonlin program, in this study the model 200 (non-compartmental analysis, extravascular) was used but this model does not compute

the volume of distribution. However in the guidelines of WinNonlin the equation for manual calculation for V_{ss} is presented:

$$V_{ss}/F = MRT_{inf} * Cl$$

*MRTinf and Cl are obtained from the estimated values from fitting each subject's data.

The comparison of V_{ss} values between these two programs is shown in Table 20. Only the racemic form showed the similar results between two programs, p-value >0.05. The p-value for R-form is 0.0104 indicating a statistically significant difference in volume of distribution values between the two programs.

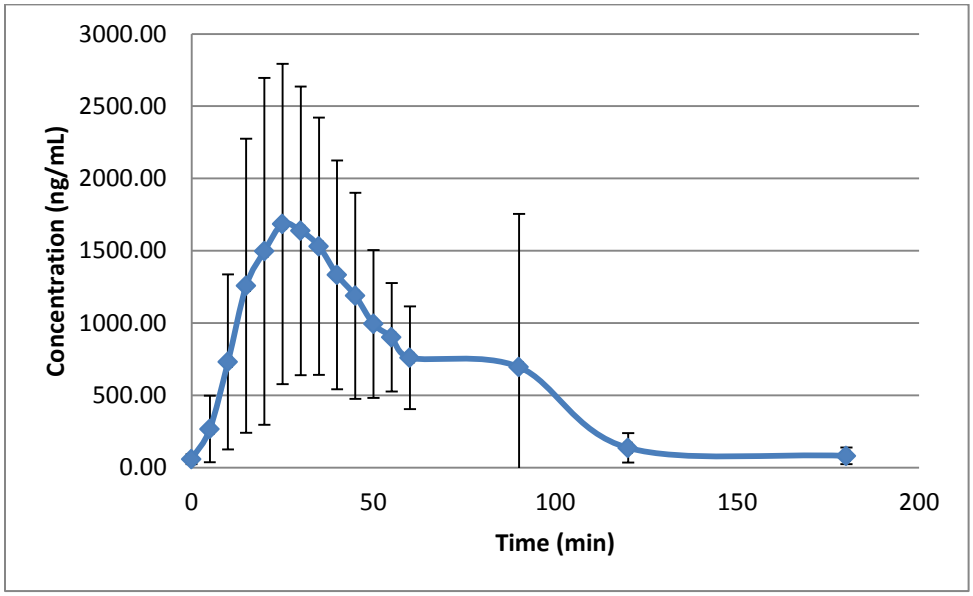

Figure 16.1 Plot of mean plasma concentrations of racemic form vs. time after administration of Lipoic acid 500 mg

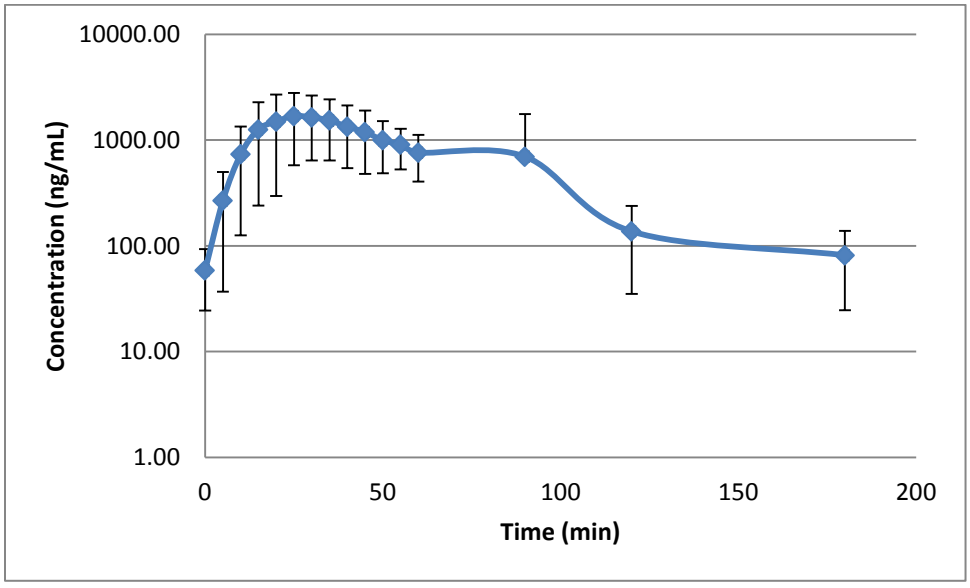

Figure 16.2 Semilogarithmic plot of mean plasma concentrations of racemic form vs. time after administration of Lipoic acid 500 mg

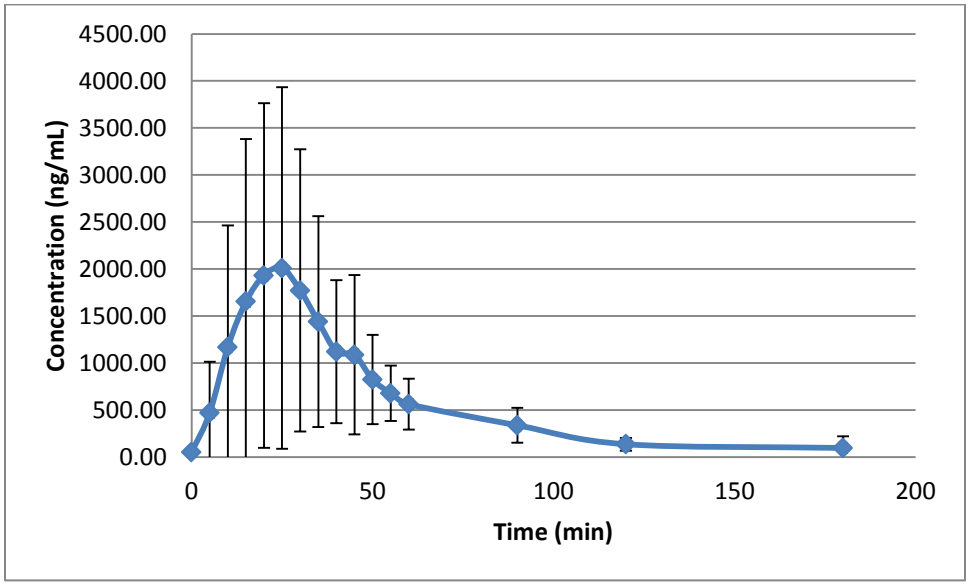

Figure 17.1 Plot of mean plasma concentrations of r-form vs. time after administration of Lipoic acid 500 mg

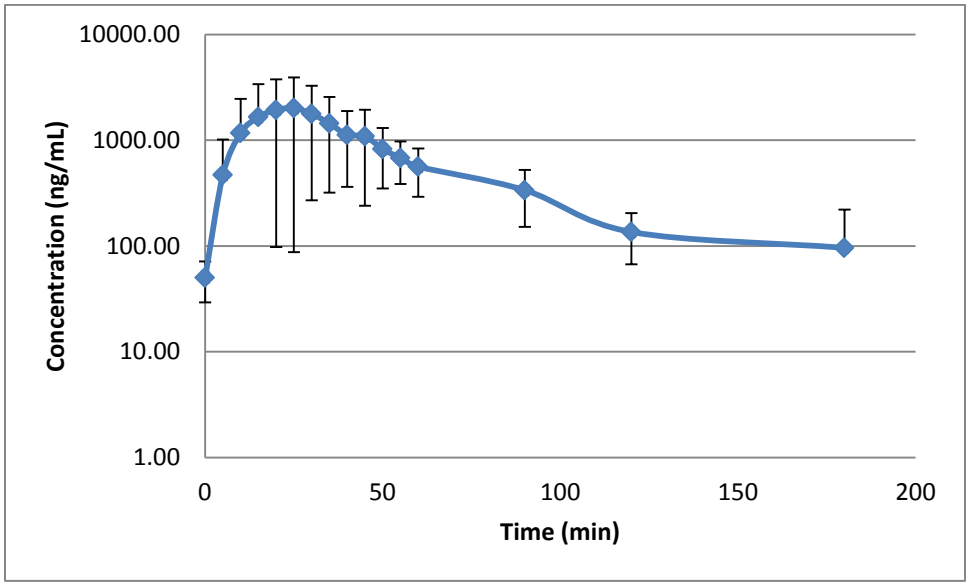

Figure 17.2 Semilogarithmic plot of mean plasma concentrations of r-form vs. time after administration of Lipoic acid 500 mg

Table 15 The Pharmacokinetic parameters of racemic form of Lipoic acid 500mg from Kinetica software

Sample	Parameter								
	C_{max} (ng/mL)	T_{max} (min)	$t_{1/2}$ (min)	k (min^{-1})	AUC ((min)*(ng/mL))	AUMC (min^2*(ng/mL))	MRT (min)	Cl/F (mL min^{-1})	V_z/F (L)
1	3143.6809	40.0000	57.8299	0.0120	134135.7402	6755590.6306	50.3638	3727.5673	310.9942
2	1513.5771	15.0000	42.3608	0.0164	53960.0879	2842467.7930	52.6772	9266.1080	566.2864
3	2839.3964	35.0000	53.2261	0.0130	122810.3257	6671136.2459	54.3206	4071.3189	312.6326
4	2451.8357	30.0000	24.0928	0.0288	99277.3149	5225263.7390	52.6330	5036.3973	175.0577
5	2079.2983	40.0000	27.2470	0.0254	94172.5868	5862206.4483	62.2496	5309.4007	208.7076
6	1170.0211	20.0000	35.3170	0.0196	41591.7635	1849685.3899	44.4724	12021.6110	612.5203
7	3715.5859	20.0000	25.1795	0.0275	104072.8972	4571345.4567	43.9245	4804.3248	174.5236
8	1292.5846	35.0000	192.0856	0.0036	168416.6570	42216586.0305	250.6675	2968.8275	822.7244
9	1254.0024	45.0000	33.3163	0.0208	87701.1879	5537829.6357	63.1443	5701.1771	274.0289
10	2032.9500	30.0000	24.4639	0.0283	101195.5374	5030085.8451	49.7066	4940.9293	174.3846
11	4333.5052	25.0000	20.8330	0.0333	157317.4742	5406443.1811	34.3665	3178.2865	95.5253
12	3788.0372	25.0000	23.8851	0.0290	145027.3201	6562451.9561	45.2498	3447.6263	118.8016
13	2291.7000	90.0000	23.2232	0.0298	109207.2944	9712476.2964	88.9361	4578.4487	153.3965
14	2551.6523	15.0000	27.6052	0.0251	108104.1734	5084198.7767	47.0306	4625.1683	184.2014
15	2119.4927	90.0000	21.9569	0.0316	134186.6464	10071856.1144	75.0586	3726.1532	118.0337
16	1249.6091	55.0000	51.7002	0.0134	77271.9817	7141726.8656	92.4232	6470.6507	482.6306
17	1274.0000	35.0000	27.2653	0.0254	77883.7538	4768813.2901	61.2299	6419.8241	252.5274
18	2229.7826	35.0000	26.9232	0.0257	130754.1168	6553253.0396	50.1189	3823.9714	148.5307
19	4226.2000	90.0000	19.5598	0.0354	155014.3674	13992555.7654	90.2662	3225.5075	91.0200
mean	2397.732	40.526	29.641	0.023	110636.907	8202945.921	68.886	4519.288	277.712
SD	1035.189	24.204	38.620	0.008	34610.243	8661350.798	47.070	2246.372	201.891

* Values were calculated using harmonic mean.

Table 16 The Pharmacokinetic parameters of r-form of Lipoic acid 500mg from Kinetica software

Sample	Parameter								
	C_{max} (ng/mL)	T_{max} (min)	$t_{1/2}$ (min)	k (min^{-1})	AUC ((min)*(ng/mL))	AUMC (min^2*(ng/mL))	MRT (min)	Cl/F (mL min^{-1})	V_z/F (L)
1	2943.8595	35.0000	23.2082	0.0299	118416.4863	6235168.3142	52.6546	4222.3850	141.3753
2	743.1164	30.0000	36.3166	0.0191	49833.6817	3636659.5800	72.9759	10033.3747	525.6866
3	1035.0658	35.0000	36.1850	0.0192	76081.8785	5381106.2770	70.7278	6571.8672	343.0776
4	1887.7893	20.0000	31.0105	0.0224	104024.8363	5493573.2064	52.8102	4806.5445	215.0382
5	1613.9489	25.0000	80.7329	0.0086	56884.8974	3011360.7875	52.9378	8789.6792	1023.7601
6	926.4424	25.0000	39.6621	0.0175	32128.8707	1509224.7785	46.9741	15562.3273	890.4816
7	952.7529	25.0000	26.1233	0.0265	60421.3144	4347158.6959	71.9474	8275.2255	311.8760
8	840.9543	35.0000	76.9605	0.0090	87163.7920	11818186.1743	135.5860	5736.3268	636.9070
9	3564.0058	30.0000	48.5236	0.0143	137379.0378	7118324.7165	51.8152	3639.5655	254.7871
10	619.2500	60.0000	35.9632	0.0193	47802.2486	4352455.6424	91.0513	10459.7590	542.6933
11	2644.5240	15.0000	8.8000	0.0788	134657.8085	9558733.8463	70.9854	3713.1155	263.5769
12	8438.0723	25.0000	111.8873	0.0062	277314.0066	13096250.6363	47.2253	1803.0103	291.0405
13	524.9500	90.0000	36.1308	0.0192	49276.5596	4283082.6955	86.9193	10146.8123	528.9099
14	2692.4768	15.0000	26.3175	0.0263	96311.3360	3613680.2902	37.5208	5191.4969	197.1115
15	1347.1464	30.0000	26.5631	0.0261	79734.7986	4161563.9791	52.1926	6270.7878	240.3118
16	1478.6617	15.0000	36.3309	0.0191	90056.6946	5468443.2290	60.7222	5552.0581	291.0075
17	3080.8000	20.0000	39.0421	0.0178	121076.2017	5810808.7062	47.9930	4129.6307	232.6047
18	3383.0175	20.0000	31.5741	0.0220	164586.9837	8119943.8579	49.3353	3037.9073	138.3820
19	5492.4000	20.0000	28.9999	0.0239	171840.5076	6908603.2085	40.2036	2909.6748	121.7348
mean	2326.802	30.000	30.996	0.022	102894.313	5996017.296	62.767	4859.355	378.440
SD	1971.669	17.873	24.044	0.015	58270.235	2949658.485	23.064	3427.236	252.304

* Values were calculated using harmonic mean.

Table 17 The Pharmacokinetic parameters of racemic form of Lipoic acid 500mg from WinNonlin software

Sample	Parameter								
	C_{max} (ng/mL)	T_{max} (min)	$t_{1/2}$ (min)	k (min^{-1})	AUC ((min)*(ng/mL))	AUMC (min^2*(ng/mL))	MRT (min)	Cl/F (mL min^{-1})	V_z/F (L)
1	3143.6809	40.0000	57.1641	0.0121	134092.0203	6739153.3302	50.2577	3728.7827	307.5138
2	1513.5771	15.0000	47.3035	0.0147	54306.0749	2949501.6953	54.3126	9207.0731	628.3318
3	2839.3964	35.0000	54.3037	0.0128	122902.3520	6701977.2538	54.5309	4068.2704	318.7232
4	2451.8357	30.0000	31.5454	0.0220	99757.6618	5350789.6331	53.6379	5012.1463	228.1047
5	2451.8357	30.0000	31.5454	0.0220	99757.6618	5350789.6331	53.6379	5012.1463	228.1047
6	1170.0211	20.0000	39.9648	0.0173	41723.7260	1887016.6950	45.2265	11983.5894	690.9386
7	3715.5859	20.0000	48.8613	0.0142	106649.7665	5319923.5364	49.8822	4688.2428	330.4836
8	1292.5846	35.0000	183.0674	0.0038	164670.6868	39574330.3810	240.3241	3036.3631	801.9351
9	1254.0024	45.0000	45.3199	0.0153	89435.9519	6035885.3779	67.4884	5590.5929	365.5283
10	2032.9500	30.0000	44.3479	0.0156	102521.7070	5400376.9952	52.6754	4877.0159	312.0341
11	4333.5052	25.0000	107.2655	0.0065	163006.6476	7481893.8074	45.8993	3067.3596	474.6784
12	3788.0372	25.0000	34.4953	0.0201	145911.1799	6796538.8364	46.5800	3426.7422	170.5357
13	2291.7000	90.0000	46.1857	0.0150	113139.5223	10795598.9890	95.4185	4419.3222	294.4680
14	2551.6523	15.0000	95.4381	0.0073	115124.5837	7598551.0905	66.0029	4343.1210	597.9961
15	2119.4927	90.0000	46.2134	0.0150	137351.5219	10951475.1979	79.7332	3640.2946	242.7054
16	1249.6091	55.0000	53.4231	0.0130	77628.5048	7242144.4885	93.2923	6440.9330	496.4240
17	1274.0000	35.0000	30.4792	0.0227	78265.2831	4822656.5909	61.6194	6388.5286	280.9172
18	2229.7826	35.0000	30.3591	0.0228	131017.0990	6621989.9762	50.5429	3816.2958	167.1498
19	4226.2000	90.0000	35.4346	0.0196	158012.9046	14761498.7952	93.4196	3164.2985	161.7632
mean	2417.339	40.000	45.154	0.015	112382.887	8546425.911	71.289	4449.076	373.597
SD	1032.347	24.324	36.936	0.005	34782.421	8048265.322	44.184	2246.363	188.690

* Values were calculated using harmonic mean.

Table 18 The Pharmacokinetic parameters of r-form of Lipoic acid 500mg from WinNonlin software

Sample	Parameter								
	C_{max} (ng/mL)	T_{max} (min)	$t_{1/2}$ (min)	k (min^{-1})	AUC ((min)*(ng/mL))	AUMC (min^2*(ng/mL))	MRT (min)	Cl/F (mL min^{-1})	V_z/F (L)
1	2943.8595	35.0000	55.7585	0.0124	121151.6594	7039103.4571	58.1016	4127.0586	331.9908
2	743.1164	30.0000	36.0201	0.0192	49820.7249	3631509.6165	72.8915	10035.9840	521.5301
3	1035.0658	35.0000	46.2685	0.0150	77013.8433	5654605.1551	73.4232	6492.3393	433.3725
4	1887.7893	20.0000	39.0436	0.0178	104723.5656	5689962.6329	54.3332	4774.4746	268.9363
5	1613.9489	25.0000	86.4361	0.0080	57088.6928	3097194.1031	54.2523	8758.3018	1092.1689
6	926.4424	25.0000	38.3644	0.0181	32071.5595	1500542.7506	46.7873	15590.1368	862.8841
7	952.7529	25.0000	31.4983	0.0220	60534.3923	4394252.9033	72.5910	8259.7674	375.3435
8	840.9543	35.0000	110.6477	0.0063	98015.5064	16715954.3567	170.5440	5101.2337	814.3147
9	3564.0058	30.0000	48.8619	0.0142	137412.1278	7128929.9951	51.8799	3638.6890	256.5013
10	619.2500	60.0000	42.8743	0.0162	48489.3177	4551792.9936	93.8721	10311.5495	637.8165
11	2644.5240	15.0000	8.8000	0.0788	134657.8085	9558733.8463	70.9854	3713.1155	263.5769
12	8438.0723	25.0000	122.5794	0.0057	278506.9282	13747603.1795	49.3618	1795.2875	317.4871
13	524.9500	90.0000	68.5866	0.0101	53012.9760	5513706.4838	104.0067	9431.6531	933.2582
14	2692.4768	15.0000	122.6120	0.0057	104148.5823	6708584.3505	64.4136	4800.8335	849.2278
15	1347.1464	30.0000	35.4505	0.0196	80082.7918	4267919.4813	53.2938	6243.5386	319.3216
16	1478.6617	15.0000	65.5080	0.0106	94314.5352	6865948.7449	72.7984	5301.4098	501.0259
17	3080.8000	20.0000	39.7524	0.0174	121114.8000	5826368.1851	48.1062	4128.3146	236.7615
18	3383.0175	20.0000	38.4754	0.0180	165400.9599	8348972.4672	50.4772	3022.9571	167.7993
19	5492.4000	20.0000	89.5506	0.0077	179249.5569	9518337.1377	53.1010	2789.4072	360.3754
mean	2326.802	30.000	40.821	0.017	105095.280	6829474.834	69.222	4757.588	502.300
SD	1971.669	17.873	32.562	0.016	58602.430	3639220.371	29.101	3412.106	278.095

* Values were calculated using harmonic mean.

Table 19 A comparison of Pharmacokinetic parameter between Kinetica and WinNonlin software

Parameter	Unit	Racemic form			R-form		
		Kinetica	WinNonlin	p-value	Kinetica	WinNonlin	p-value
C_{max}	ng/mL	2397.732	2417.339	0.3306	2326.802	2326.802	N/A
T_{max}	min	40.526	40.000	0.3306	30.000	30.000	N/A
$t_{1/2}$	min	29.641	45.154	0.0083*	30.996	40.821	0.0051*
k	min^{-1}	0.023	0.015	0.0003*	0.022	0.017	0.0017*
AUC	(min)*(ng/mL)	110636.907	112382.887	0.0078*	102894.313	105095.280	0.0079*
AUMC	min^2*(ng/mL)	8202945.921	8546425.911	0.1645	5996017.296	6829474.834	0.0138*
MRT	min	68.886	71.289	0.1124	62.767	69.222	0.0118*
Cl/F	mL min^{-1}	4519.288	4449.076	0.0021*	4859.355	4757.588	0.0153*
V_z/F	L	277.712	373.597	0.0023*	378.440	502.300	<0.005*

*statistically significant

Table 20 A comparison of Volume of distribution (V_{ss}) of Lipoic acid between Kinetica and WinNonlin software

Subject	Racemic form		R-from	
	Kinetica	WinNonlin	Kinetica	WinNonlin
1	187.7346	187.4000	222.3278	239.7887
2	488.1129	500.0601	732.1949	731.5379
3	221.1567	221.8464	464.8139	476.6883
4	265.0807	268.8410	253.8346	259.4125
5	330.5081	268.8410	465.3062	475.1580
6	534.6298	541.9758	731.0263	729.4204
7	211.0274	233.8599	595.3813	599.5848
8	744.1886	729.7112	777.7654	869.9848
9	359.9968	377.3002	188.5849	188.7748
10	245.5968	256.8988	952.3744	967.9668
11	109.2264	140.7897	263.5769	263.5769
12	156.0043	159.6177	85.1478	88.6186
13	407.1896	421.6851	881.9535	980.9551
14	217.5242	286.6586	194.7892	309.2390
15	279.6797	290.2523	327.2885	332.7419
16	598.0385	600.8895	337.1333	385.9342
17	393.0851	393.6573	198.1933	198.5975
18	191.6533	192.8867	149.8760	152.5904
19	291.1543	295.6075	116.9793	148.1203
mean	327.9783	335.1989	417.8183	442.0364
SD	165.5725	160.4781	277.9641	288.7714
p-value	0.2052		0.0104*	

*statistically significant

DISCUSSION

The pharmacokinetic parameter results of two programs show that two forms of lipoic acid (racemic and R- form) have the same rate of absorption and almost the same elimination rate. However this study did not measure the concentrations versus time for each enantiomer (R- and S- form) of lipoic acid.

The T_{max} and $t_{1/2}$ values are comparable to the previous study's results.[22] In that study, the drug was given to the fasting healthy volunteers. The concentration of both enantiomers increased rapidly within 0.5-1 hour. The half-life was between 0.47-0.64 hours, the same as our results, 0.494-0.753 hour. The clearance of racemic form of lipoic acid was a little higher than the R-form of lipoic acid. However, this result could not confirm that the differences come from the different clearance rates of R- and S- forms of lipoic acid since S-lipoic acid clearance was not measured.

Unlike xanthohumol, many pharmacokinetic parameter of lipoic acid obtained from Kinetica and WinNonlin were statistically different ($t_{1/2}$, k, AUC, MRT, Cl/F and V_{ss}). First, the T_{max} values of racemic form of lipoic acid are different, 40.526 and 40.000 minutes for Kinetica and WinNonlin, respectively. Even if it is not clinically significantly different, this value should be the same. The reason for the different values is subject no. 5. WinNonlin did not choose the time at the highest plasma concentration. The software selected the time point before the highest peak, 30 minutes instead of 40 minutes.

The elimination rate constant (k) values are different between Kinetica and WinNonlin in both racemic and R-forms of lipoic acid. WinNonlin program calculates the λ_z (elimination rate constant, or called "k") by choosing the last three time points first and then last four and so on. The regression with the largest adjusted R^2, the square of the correlation coefficient, is selected to estimate λ_z. The lipoic acid data sets are not normal like the xanthohumol data sets where the elimination phase is linear in the semilogarithmic scale. Kinetica and WinNonlin chose the different time points for the

elimination rate constant calculation. The difference in half-life comes from the equation that relates half-life to the elimination rate constant's value.

$$t_{1/2} = 0.693/k$$

The rest of the pharmacokinetic parameters (AUMC, MRT, Cl/F and V_z/F) utilize the AUC for their calculation. However, the percent differences between two the programs of AUC are -1.6 and -2.2% for racemic and R-form, respectively.

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CHAPTER 4

CONCLUSION

The concentration of xanthohumol increased rapidly after oral administration. The C_{max} was around 45, 66 and 133 ug/L and T_{max} was 2, 10 and 17 hours for low, medium and high dose respectively. The mean plasma concentration versus time curves after oral administration of xanthohumol showed two peaks in low and medium dose groups. The first peak comes from the dissolved xanthohumol readily absorbed into the blood circulation. After that the precipitated portion of xanthohumol slowly dissolves and produces a second drug peak. A plot of C_{max} versus dose and AUC versus dose shows the linearity of the pharmacokinetics of Xanthohumol. When the dose is increased, the C_{max} and AUC will increase in the same proportion.

For isoxanthohumol, after administration the isoxanthohumol concentration increased rapidly in both groups and reach peak concentrations in 8 and 5 hours for medium and high dose, respectively and a second peak occurred around 24 hours. Isoxanthohumol in high dose group was produced to a greater extent than the medium dose group and T_{max} in both groups of isoxanthohumol was later than xanthohumol. Both xanthohumol and isoxanthohumol had a high volume of distribution.

Alpha lipoic acid is absorbed rapidly in both forms (R- and S- form). The concentration climbs to the highest peak within 1 hour. The half-life is from 0.494 to 0.753 hour. Elimination rate constant (k) is about 0.02 min^{-1} for both racemic and R-form. Clearance value of racemic form is higher than the R-form product. The differences in pharmacokinetic parameters between two programs come from the calculation method in each software.

The comparison of pharmacokinetic results obtained from Kinetica and WinNonlin software show that only normal data sets such as for xanthohumol and isoxanthohumol are conductive for comparative analysis for by them. These two programs gave the same results for xanthohumol and isoxanthohumol but not for Lipoic acid between two pharmacokinetic programs. The pharmacokinetic results obtained from pharmacokinetic program can vary due to the different of calculation method and a variation and limitation of the program.

The calculation method of elimination constant (k) is different. WinNonlin use the largest adjusted R^2, the square of the correlation coefficient, to estimate λ_z. Moreover only Kinetica program (noncompartmental model) calculates the volume of distribution at steady state (V_{ss}). On the other hand, WinNonlin has more choices for weighting data than Kinetica. For the noncompartmental model, Kinetica software provides only uniformity weighing to fit the data set.

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APPENDICES

Table 21 Subject detail in sex for Xanthohumol and Isoxanthohumol study

Low dose		Medium dose		High dose	
No.	**Sex**	**No.**	**Sex**	**No.**	**Sex**
1	m	1	m	1	f
2	m	2	f	2	f
3	f	3	m	3	f
4	f	4	f	4	m
5	m	5	f	5	f
6	m	6	m	6	f
7	f	7	f	7	m
8	m	8	f	8	f
9	f	9	f	9	m
10	f	10	m	10	f
11	f	11	f	11	m
12	m	12	m	12	m
13	m	13	m	13	m
14	f			14	m
15	m			15	f
16	m			16	f
17	f			17	m
18	m				

* f = female, m= male

Table 22 Subject detail in sex for Lipoic acid study

Lipoic acid		
No.	**Sex**	**Age**
1	m	y
2	m	y
3	f	y
4	f	y
5	m	e
6	f	e
7	m	y
8	f	y
9	m	e
10	m	y
11	f	e
12	m	e
13	m	y
14	m	e
15	f	y
16	f	y
17	m	e
18	f	e
19	m	e

* f = female, m= male, y= young, e= elderly

Table 23 Concentration (µg/L) of *Xanthohumol in Low dose group (20mg)*

Subject	Time (hour)													
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**
1	0	0	0	34	15	6	0	0	5	0	0	0	0	0
2	0	0	53	115	16	8	11	6	3	3	4	0	0	0
3	0	0	19	11	9	4	6	5	3	0	0	0	0	0
4	0	0	0	21	4	0	0	0	0	0	0	0	0	0
5	0	0	10	84	21	10	15	4	7	0	0	0	0	0
6	0	0	0	77	64	16	20	10	0	0	0	0	0	0
7	0	18	71	32	15	15	14	7	0	0	0	0	0	0
8	0	2	21	64	70	2	21	11	2	0	0	0	0	0
9	0	0	29	23	5	3	10	4	3	2	0	0	0	0
10	0	0	21	9	3	0	4	0	0	0	0	0	0	0
11	0	0	31	10	6	5	5	3	0	0	0	0	0	0
12	0	0	0	30	14	5	5	3	0	0	0	0	0	0
13	0	0	0	3	4	0	2	0	0	0	0	0	0	0
14	0	0	11	12	4	2	2	2	2	0	0	0	0	0
15	0	0	13	7	3	0	6	3	0	0	0	0	0	0
16	0	0	21	20	6	4	6	5	3	3	0	0	0	0
17	0	0	4	36	12	6	10	6	0	0	0	0	0	0
18	0	11	53	7	4	4	5	5	2	0	0	0	0	0

Table 24 Concentration (µg/L) of *Xanthohumol in Medium dose group (60mg)*

Subject	Time (hour)													
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**
1	0	35	49	33	14	13	8	10	6	4	3	0	0	0
2	0	0	19	64	21	6	10	4	3	4	3	3	0	0
3	0	3	17	33	23	9	5	7	6	6	2	0	0	0
4	0	0	14	49	9	7	11	8	4	0	0	0	0	0
5	0	0	0	21	44	53	16	16	15	11	5	0	0	0
6	0	20	98	18	13	8	13	14	7	5	0	0	0	0
7	0	0	0	8	49	32	10	10	5	5	2	0	0	0
8	0	6	164	121	26	20	31	17	9	10	3	0	0	0
9	0	0	4	22	30	29	15	5	3	3	0	0	0	0
10	0	0	2	69	56	27	13	8	4	4	0	0	0	0
11	0	9	82	55	16	9	12	9	5	4	0	0	0	0
12	0	8	114	115	21	13	13	14	9	5	2	0	0	0
13	0	0	12	13	8	13	14	5	3	0	0	0	0	0

Table 25 Concentration (μg/L) of *Xanthohumol in High dose group (180mg)*

Subject	Time (hour)													
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**
1	0	0	5	197	79	44	43	16	16	13	6	2	0	0
2	0	0	0	165	98	61	61	32	18	17	5	0	0	0
3	0	17	306	191	104	54	39	17	12	12	7	0	0	0
4	0	0	0	0	0	0	32	28	20	12	6	0	0	0
5	0	0	0	0	90	41	53	21	12	39	14	7	0	0
6	0	31	171	189	46	29	39	33	22	8	4	0	0	0
7	0	12	152	205	59	41	100	21	18	16	4	0	0	0
8	0	9	30	112	91	42	34	17	15	13	4	0	0	0
9	0	0	0	0	25	28	29	11	12	6	2	0	0	0
10	0	0	31	173	77	37	44	13	11	15	4	0	0	0
11	0	0	0	48	80	30	137	11	19	13	4	0	0	0
12	0	0	11	98	56	32	28	18	35	11	5	1	0	0
13	0	0	0	0	0	4	18	22	10	7	3	0	0	0
14	0	0	48	51	72	65	27	25	24	15	4	0	0	0
15	0	0	0	14	9	18	12	9	7	7	0	0	0	0
16	0	0	194	352	240	153	121	59	24	19	6	0	0	0
17	0	0	21	71	38	21	38	12	10	6	0	0	0	0

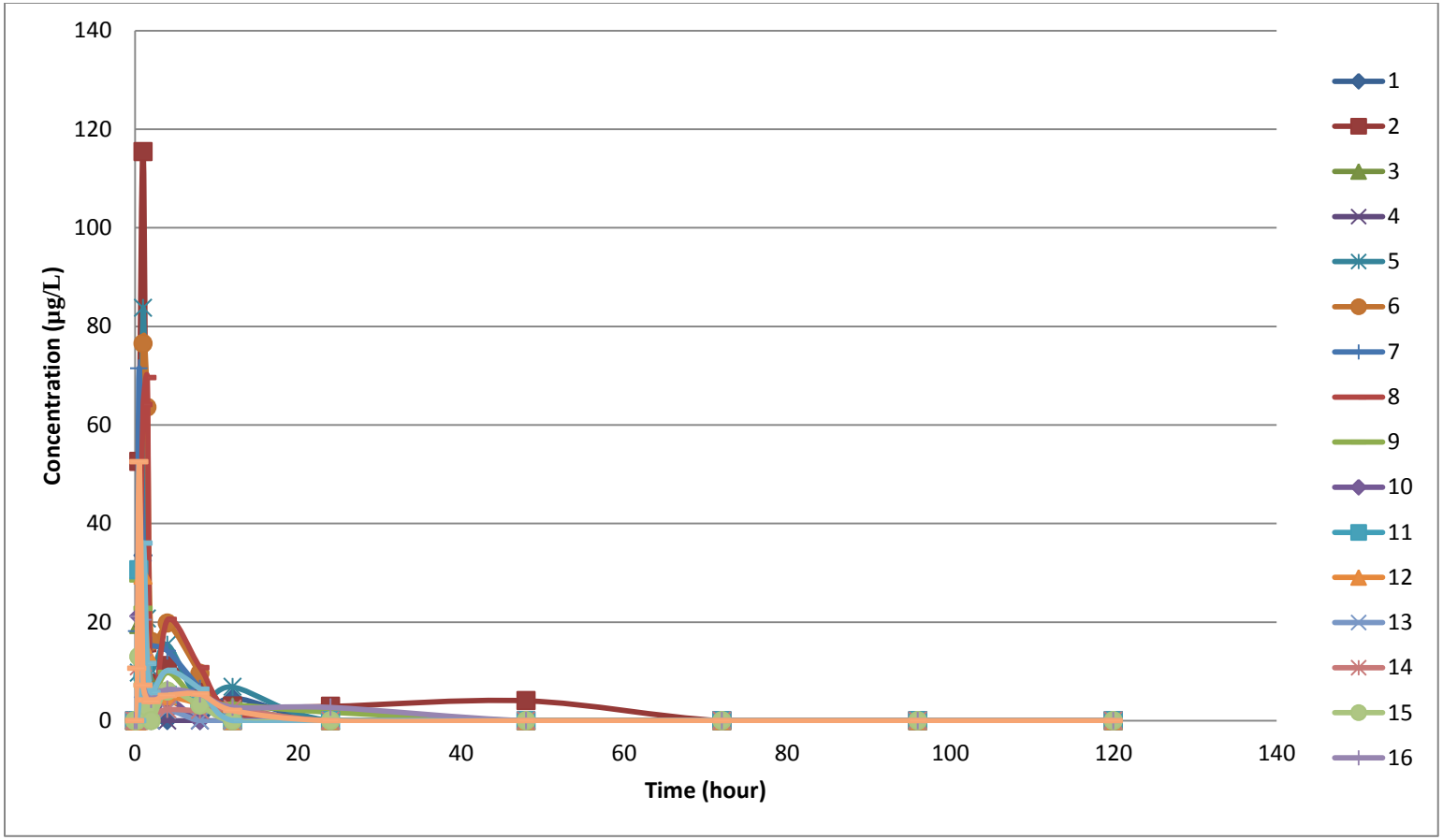

Figure 18 Scatter plot of *Xanthohumol in Low dose group (20mg)*

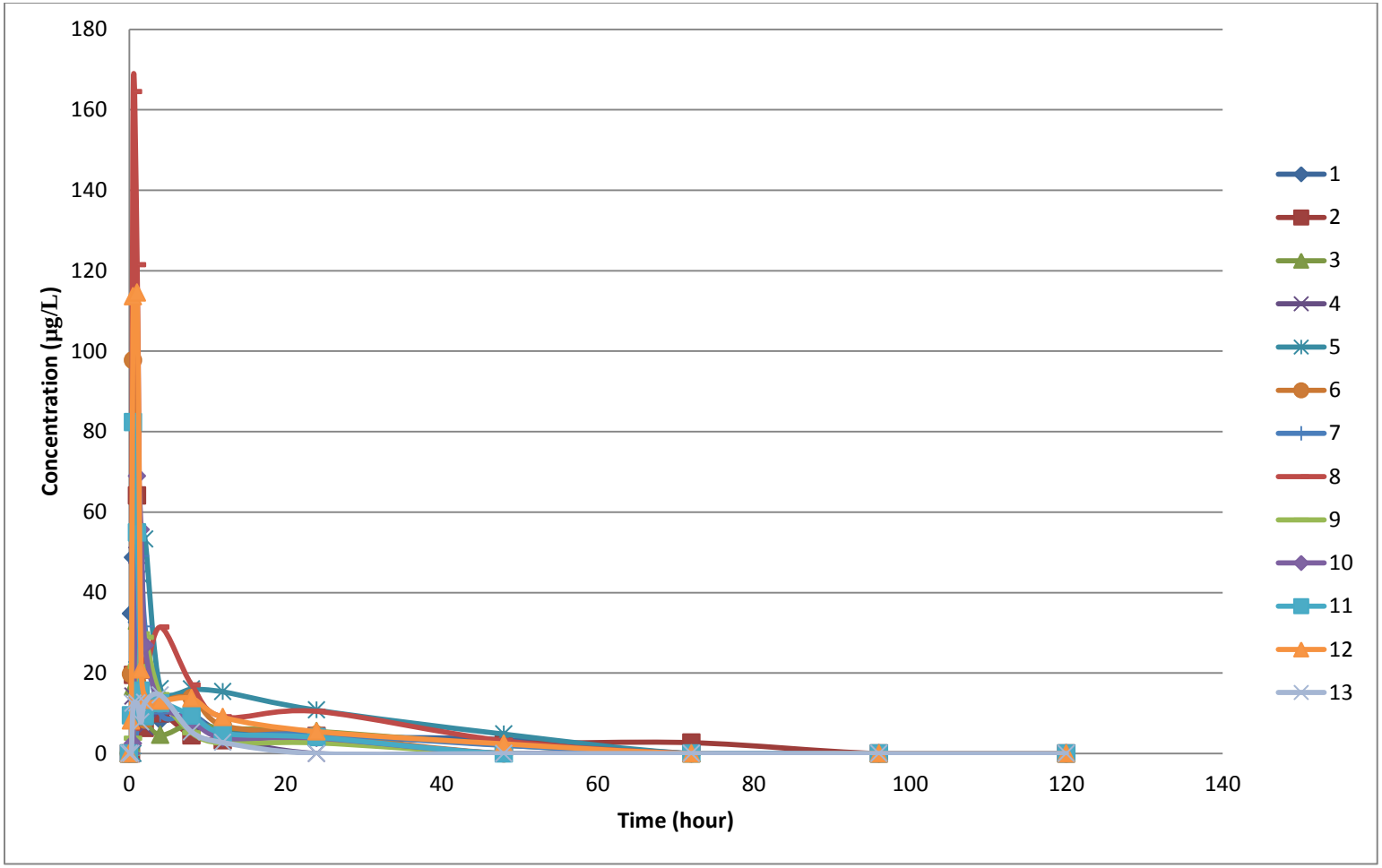

Figure 19 Scatter plot of *Xanthohumol in Medium dose group (60mg)*

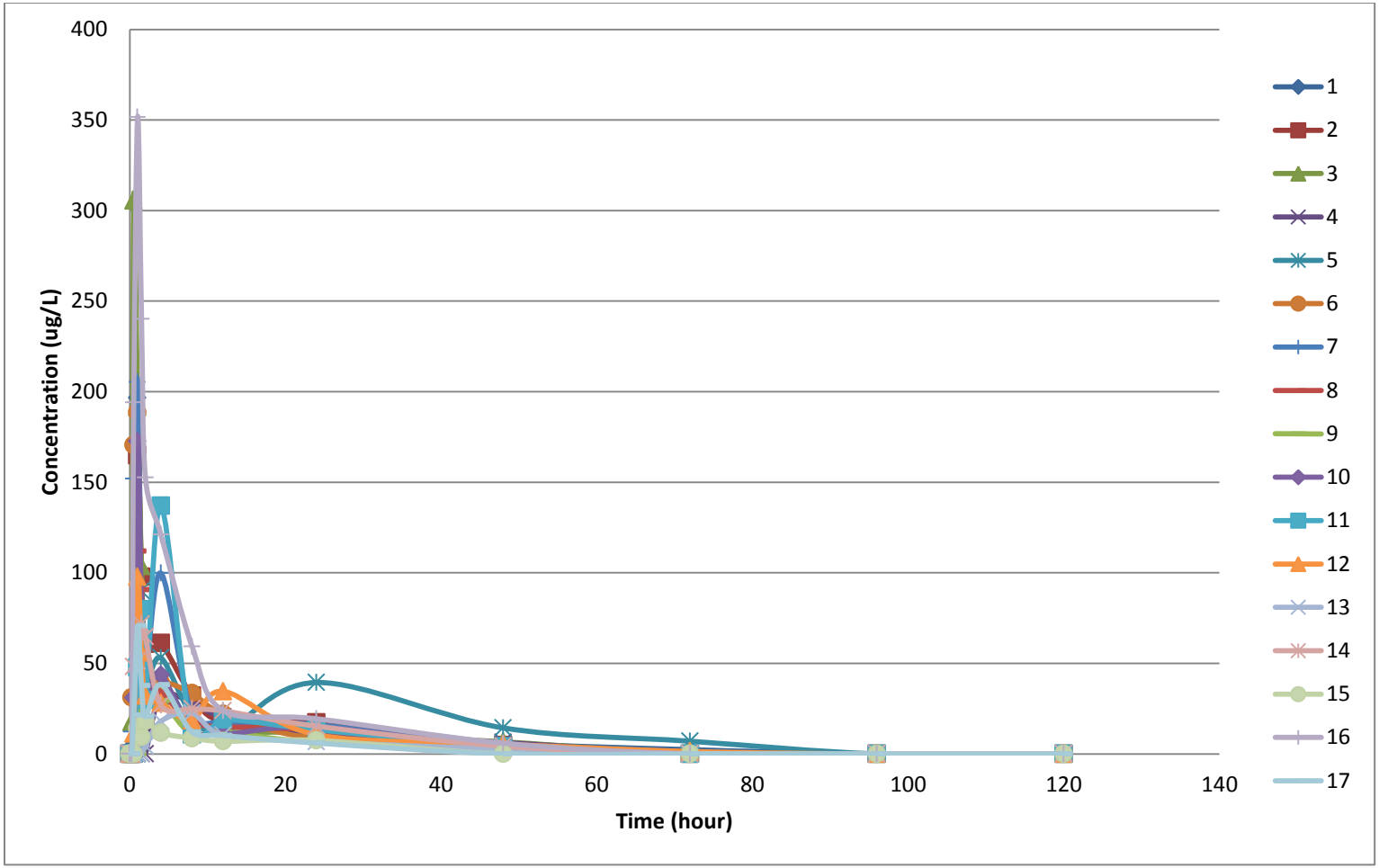

Figure 20 Scatter plot of *Xanthohumol in High dose group (180mg)*

Table 26 Concentration (μg/L) of *Isoxanthohumol in Medium dose group (60mg)*

Subject	Time (hour)													
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**
1	0	0	0	0	1	5	4	4	4	1	3	0	0	0
2	0	0	4	10	2	2	7	10	6	8	6	5	0	0
3	0	0	0	0	0	0	0	9	5	3	0	0	0	0
4	0	0	0	0	0	0	4	5	3	0	0	0	0	0
5	0	0	0	0	0	0	6	15	11	15	10	3	0	0
6	0	0	0	0	0	0	6	7	4	2	0	0	0	0
7	0	0	0	0	9	5	3	14	8	12	4	2	0	0
8	0	0	4	3	0	0	6	10	6	9	4	1	0	0
9	0	0	0	0	0	1	4	6	4	4	1	0	0	0
10	0	0	0	2	1	0	4	8	4	4	3	1	0	0
11	0	0	4	2	0	0	4	4	2	3	0	0	0	0
12	0	0	2	4	0	0	12	19	14	10	4	3	0	0
13	0	0	4	3	4	8	11	6	3	3	0	0	0	0

Table 27 Concentration (μg/L) of *Isoxanthohumol in High dose group (180mg)*

Subject	Time (hour)													
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**
1	0	0	0	28	11	13	44	26	24	25	10	0	0	0
2	0	0	0	3	4	3	42	30	27	27	12	1	0	0
3	0	5	7	5	3	3	28	19	18	20	11	0	0	0
4	0	0	0	0	0	0	5	25	15	16	9	2	0	0
5	0	0	0	0	7	5	29	16	9	13	25	12	7	3
6	0	2	6	7	3	2	18	66	47	22	5	1	0	0
7	0	0	6	6	7	6	61	13	18	21	5	0	0	0
8	0	0	0	11	13	14	28	26	22	20	7	4	0	0
9	0	0	0	0	2	2	10	6	6	5	3	0	0	0
10	0	0	3	8	4	6	25	9	7	12	6	0	0	0
11	0	0	0	6	3	0	23	6	12	10	5	0	0	0
12	0	0	0	5	3	2	12	16	21	9	5	2	0	0
13	0	0	0	0	0	0	0	19	17	5	0	0	0	0
14	0	0	3	4	0	0	3	11	8	9	3	0	0	0
15	0	0	0	0	2	2	5	3	3	4	0	0	0	0
16	0	0	33	69	42	31	65	36	26	26	8	0	0	0
17	0	0	0	2	0	0	27	12	12	11	3	2	0	0

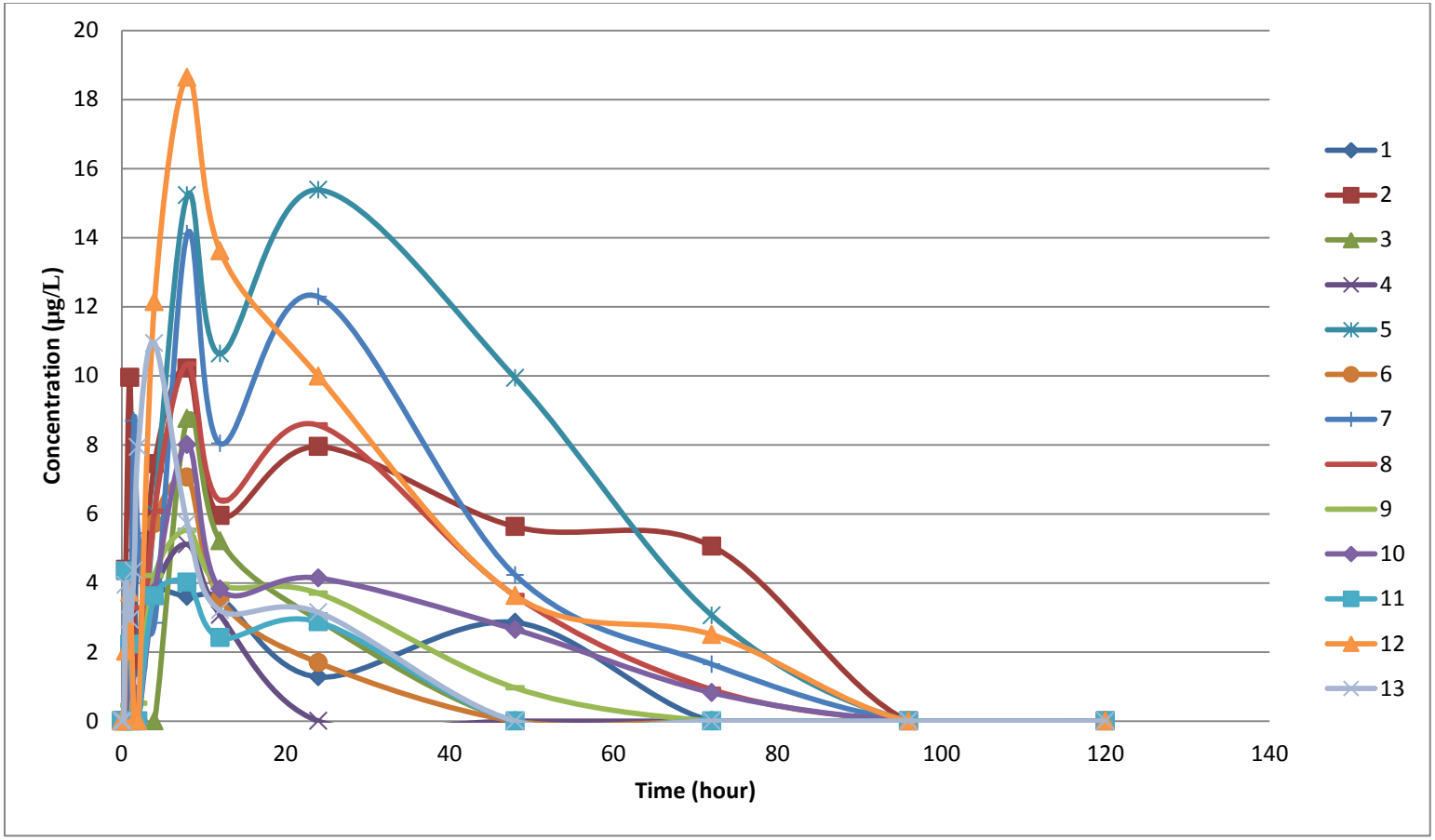

Figure 21 Scatter plot of *Isoxanthohumol in Medium dose group (60mg)*

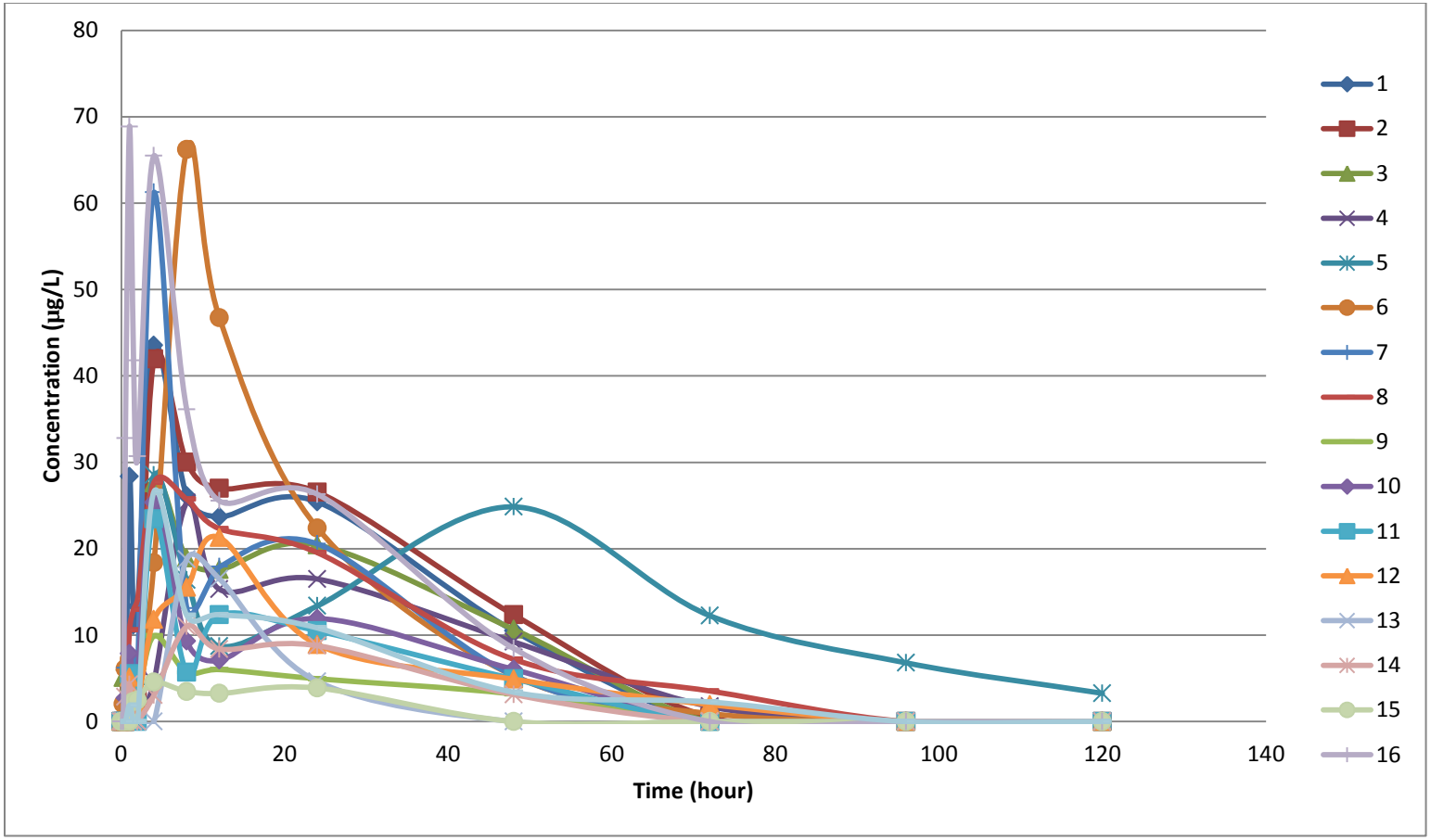

Figure 22 Scatter plot of *Isoxanthohumol in High dose group (180mg)*

Table 28 Concentration (ng/ml) of *Racemic form of Lipoic acid*

Subject	Time (min)															
	0	5	10	15	20	25	30	35	40	45	50	55	60	90	120	180
1	46.481	74.418	414.306	1699.106	2202.216	2086.988	2729.433	3080.221	3143.681	3012.458	1989.494	1280.901	842.125	140.158	103.526	48.200
2	61.707	272.620	969.551	1513.577	1378.161	1083.957	724.165	621.892	420.037	370.142	308.604	267.115	245.155	102.116	64.491	48.521
3	81.139	180.695	579.712	963.387	1761.565	2310.202	2622.698	2839.396	2220.179	2070.846	1573.695	1208.838	958.806	194.328	120.971	59.195
4	49.246	99.271	266.608	454.678	648.408	2399.122	2451.836	2130.069	1573.542	1714.205	1325.967	1084.481	620.954	448.027	72.596	45.142
5	28.426	99.531	262.869	323.842	566.391	983.071	1374.820	1399.907	2079.298	1759.044	1789.618	1154.580	1214.492	239.619	100.860	92.142
6	12.420	325.433	747.981	909.062	1170.021	1082.098	658.533	608.154	349.760	260.566	247.168	219.987	176.390	56.500	66.923	20.337
7	26.950	47.659	534.185	2596.106	3715.586	2703.940	2030.439	1115.252	1603.625	1150.613	1002.883	747.507	609.402	75.376	62.894	78.060
8	60.806	251.101	552.635	587.059	1043.316	1284.806	1171.830	1292.585	640.833	747.515	450.210	800.298	449.647	379.371	438.738	279.652
9	113.990	588.484	810.972	1156.833	1145.120	971.291	931.990	940.413	732.803	1254.002	923.769	1149.673	684.120	406.193	91.659	99.577
10	105.250	490.350	818.400	824.500	986.050	1900.400	2032.950	1883.550	1935.450	1656.200	1406.150	1091.250	797.600	218.350	103.450	46.250
11	28.224	865.829	2502.175	3997.325	4035.303	4333.505	4013.950	2998.998	2282.328	1524.520	877.762	874.474	605.547	89.734	57.594	45.624
12	19.869	225.212	945.676	2253.639	3724.320	3788.037	2959.714	2475.474	1747.083	1421.000	1044.530	1013.699	1126.061	481.705	61.466	58.063
13	81.750	86.600	109.950	122.950	237.700	342.150	257.200	382.450	412.450	220.250	268.050	190.000	1231.600	2291.700	216.850	116.550
14	57.067	230.066	1627.776	2551.652	1979.033	2007.961	1591.922	1846.534	1529.683	1212.627	1303.299	913.928	715.357	151.610	96.339	71.928
15	132.330	192.312	167.120	368.844	605.728	758.082	898.625	844.364	813.450	932.769	1168.268	1474.731	1559.075	2119.493	170.903	90.342
16	40.359	161.745	299.221	525.981	500.712	629.878	759.043	587.485	611.480	402.500	583.420	1249.609	548.612	479.296	223.654	121.257
17	32.250	66.100	303.750	687.150	249.050	774.150	1253.850	1274.000	1207.750	1151.250	1029.750	863.250	842.550	463.850	94.200	43.000
18	41.447	685.670	1591.701	1907.828	2088.074	2173.430	2122.226	2229.783	1538.294	1362.076	1107.081	1092.882	879.626	651.972	118.210	52.950
19	94.100	125.750	391.350	447.550	393.450	400.000	539.600	534.850	484.350	370.850	477.700	456.650	334.600	4226.200	331.600	129.450

Table 29 Concentration (ng/ml) of *R- form of Lipoic acid*

Subject	Time (min)															
	0	5	10	15	20	25	30	35	40	45	50	55	60	90	120	180
1	50.051	65.485	206.492	524.265	1003.369	1955.671	2204.782	2943.859	2897.747	2427.598	1925.628	1061.042	1035.889	301.004	100.483	58.245
2	59.336	35.969	51.865	124.291	376.202	503.927	743.116	719.738	604.957	527.253	499.620	474.075	451.136	357.268	121.208	41.634
3	35.845	46.193	180.183	417.280	661.738	1034.586	973.405	1035.066	825.059	766.338	663.658	645.629	638.268	675.926	121.936	64.328
4	35.091	611.161	1277.132	1785.982	1887.789	1863.723	1563.466	1427.450	1009.068	974.292	862.152	818.053	604.546	454.859	139.372	60.291
5	26.237	79.111	488.708	712.840	1347.249	1613.949	1182.138	980.161	936.435	888.347	586.989	421.434	268.695	55.021	37.451	24.768
6	14.326	70.291	182.849	432.098	694.194	926.442	678.972	349.684	409.769	598.333	356.558	416.844	200.776	49.749	32.850	10.548
7	55.553	99.599	259.773	387.626	581.098	952.753	755.854	681.467	424.601	424.771	481.244	354.965	365.209	590.896	174.677	49.852
8	91.197	270.118	278.654	393.512	548.554	710.821	673.801	840.954	599.074	469.714	673.045	687.210	365.509	289.148	217.481	223.915
9	70.257	203.234	1170.286	1386.682	2741.775	3528.526	3564.006	2373.881	1631.058	1455.990	1299.550	1223.619	916.776	246.156	157.499	67.812
10	65.950	62.800	61.650	85.900	112.050	109.600	181.550	266.600	364.800	316.300	257.200	584.600	619.250	444.600	158.350	68.100
11	15.616	1657.571	2423.729	2644.524	2246.888	1894.798	1550.514	1089.732	790.459	258.839	453.308	424.559	111.377	531.820	332.039	576.105
12	18.718	1156.248	5247.996	6219.311	7044.938	8438.072	6315.854	4881.821	2876.427	3648.103	1595.454	1034.108	832.088	147.997	106.061	82.284
13	59.550	128.900	184.000	351.900	374.050	436.600	486.400	373.700	357.350	297.700	292.900	262.750	268.250	524.950	131.200	79.400
14	50.210	1713.923	2099.297	2692.477	2539.549	2077.927	1518.687	1301.182	956.717	617.423	383.909	418.918	301.088	104.379	71.287	56.423
15	63.187	417.451	856.540	935.832	1139.893	965.031	1347.146	1200.752	787.443	1018.441	730.375	1092.836	868.434	275.161	89.596	32.857
16	55.038	591.394	1023.694	1478.662	1432.416	1300.089	1116.804	937.686	1121.887	1297.989	1004.505	636.781	582.725	241.143	122.012	101.732
17	75.150	222.250	2257.550	2572.900	3080.800	2465.950	1942.300	1393.450	1352.550	1339.100	965.350	480.750	648.300	289.400	162.000	58.000
18	49.226	1099.366	2014.028	3061.213	3383.017	2730.008	2884.181	2615.172	1966.453	1593.863	1386.574	1052.094	866.674	565.075	205.101	82.107
19	65.800	373.750	1932.400	5193.950	5492.400	4673.350	3981.150	1955.050	1388.950	1744.350	1240.000	778.200	722.050	248.650	94.300	85.200

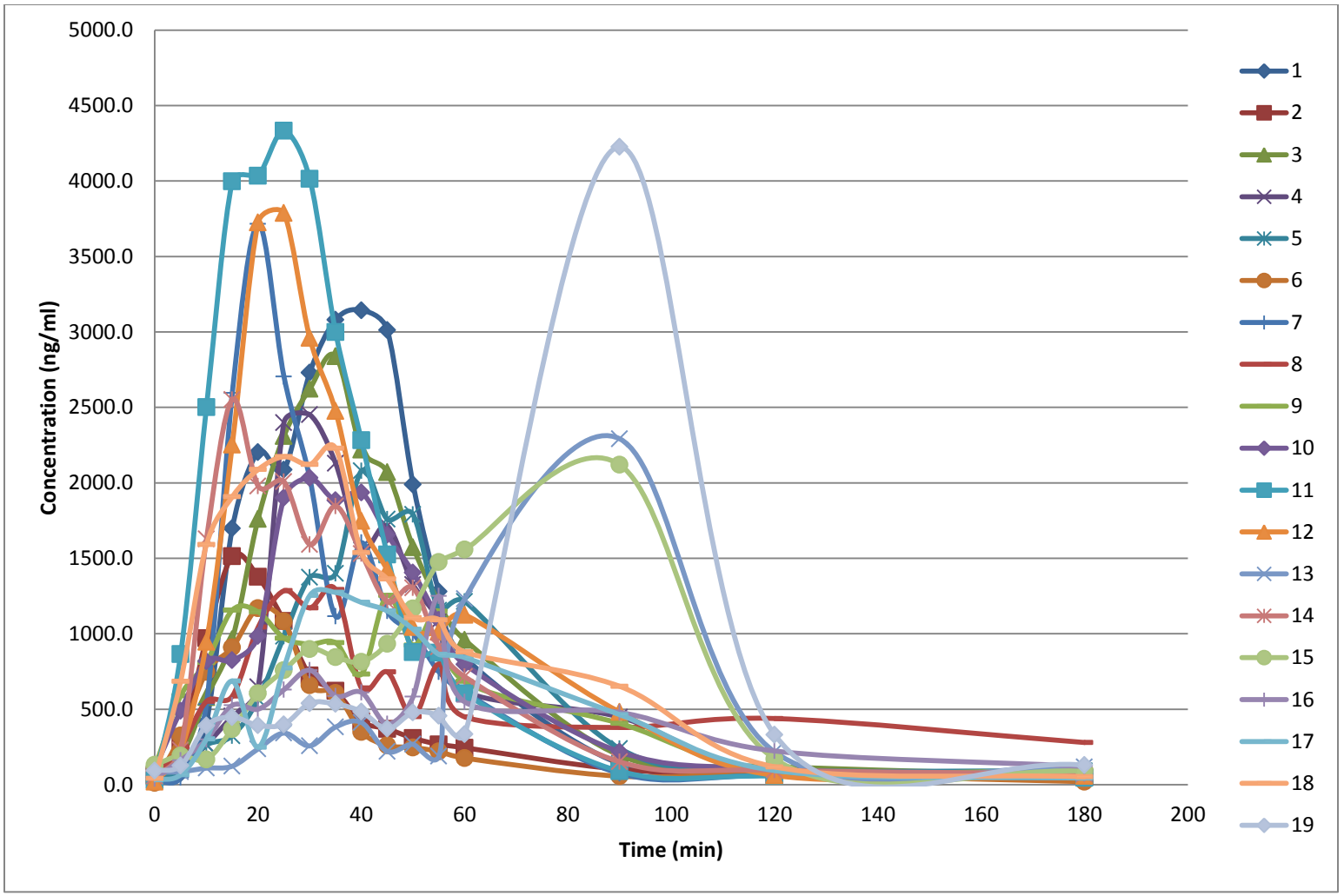

Figure 23 Scatter plot of *Racemic form of Lipoic acid*

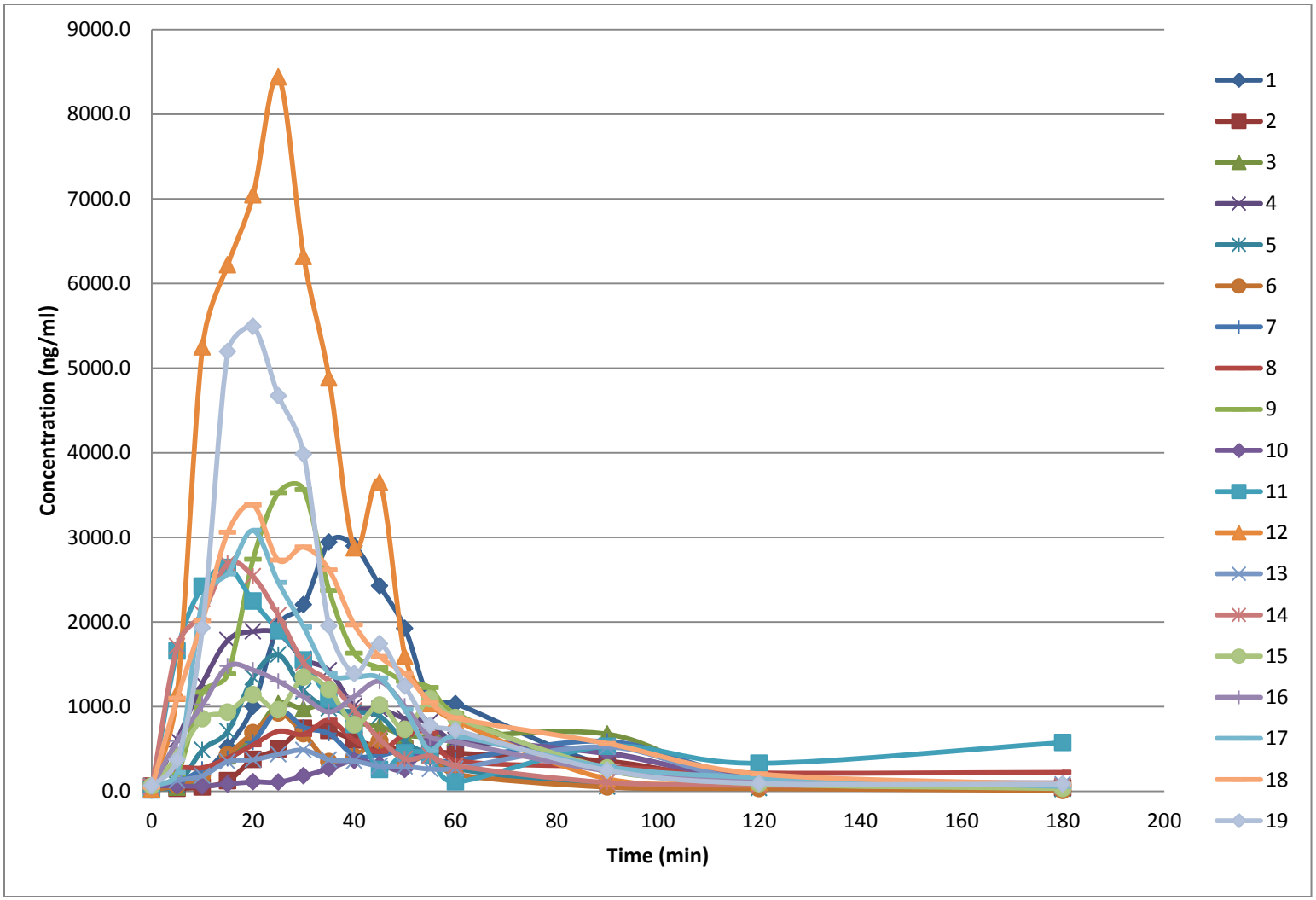

Figure 24 Scatter plot of *R- form of Lipoic acid*