

## The Pharmacokinetic Drug-Drug Interactions of *Andrographis paniculata* and Ibuprofen in the Plasma of Healthy *Oryctolagus cuniculus* Rabbits

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### Abstract

An HPLC method was developed and validated for the pharmacokinetic drug-drug interaction between *Andrographis paniculata* and ibuprofen in the plasma of *Oryctolagus cuniculus* rabbits after a single oral administration of the mixture. Nine healthy rabbits (6 males and 3 females, weight 1.68-2.42 kg) were acclimatized for 7 days and were randomly divided into 3 groups. At day-8th the rabbits were group (1) treated with a single oral administration of ibuprofen (dose of 28 mg/kg BW); group (2) treated with a single oral administration of *Andrographis paniculata* infusion (7.04 mL/kg BW); group (3) treated with a single oral administration of a mixture of *Andrographis paniculata* (7.04 mL/kg BW) infusion and ibuprofen (dose of 28 mg/kg BW). Plasma samples were prepared by collecting the blood from the marginal ear vein at 0, 30, 60, 90, and 120 minutes after the mixture administration, followed by centrifuging it for 30 minutes 3000 rpm. Chromatographic separation was performed on a LiChrosorb RP-18 with methanol and double-distilled water (70:30) as the mobile phase, flow rate 1 mL/minute. UV detection was set at 227 nm. The absorption and distribution of ibuprofen were fast ( $T_{max}$  = 30 min;  $C_{max}$  = 4.02962 mcg/mL), however, interestingly this drug could improve the absorption and distribution of andrographolide in *Oryctolagus cuniculus* rabbits.

**Keywords:** *Andrographis paniculata*, andrographolide, antiinflammation, ibuprofen, NSAIDs

### Introduction

The bioavailability of a drug is defined as its amount and rate in the systemic circulation. Once entering the blood, the concentration of a drug will denote its total concentration in the circulatory system.<sup>1</sup>

The bioavailability of a drug can be interfered with by another drug when both drugs are administered at the same time due to the occurrence of pharmacokinetic drug-drug interactions (DDIs). Many pharmacokinetic DDIs studies in humans have been reported

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underlying the mechanisms for DDIs of different degrees.<sup>2-6</sup>

Andrographolide has been reported in possessing anti-inflammatory activity among its other pharmacological activities.<sup>7-11</sup> A previous bioavailability study of sambiloto (*Andrographis paniculata*) infusion, determined as andrographolide (Figure 1a), reported that this lactone diterpene compound was absorbed and quantified in the plasma of New Zealand male rabbits at 1.5h ( $C_{max} = 0.2136 \mu\text{g/mL}$ ) after a single oral administration.<sup>12</sup> A bioavailability of andrographolide in a mixture of ethanolic extracts of sambiloto and turmeric in rabbits has been reported. In this study, andrographolide was absorbed and distributed in blood within 60-90 minutes ( $C_{max} = 3.06 - 4.41 \text{ ppm}$ ).<sup>13</sup> However, there is a very limited report of its pharmacokinetic DDIs study with NSAIDs. This work aimed to study the pharmacokinetic DDIs of *Andrographis paniculata* infusion and ibuprofen (Figure 1b) in the plasma of healthy *Oryctolagus cuniculus* rabbits.

## Methods

### Chemicals and Plants

Andrographolide 98% 500 mg CAS 5508-58-7 for R & D use (Aldrich) (Figure 1a), ibuprofen pharmaceutical grade (CV Agung Menara Abadi, Bandung) (Figure 1b),

chloroform (Merck), double-distilled water for HPLC (PT IPHA), methanol for HPLC (JT Baker), dried sambiloto (*Andrographis paniculata*) herbs and the fresh plants were obtained from Kebun Percobaan Manoko Lembang. The plants were taxonomically identified at Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (Letter No. 419/HB/05/2016).

### Identification of Ibuprofen and Andrographolide

Each 2-5 mg of ibuprofen and andrographolide was dispersed in 195-198 mg of potassium bromide crystals. The pellets were measured by using the FT-IR instrument (Shimadzu IR Prestige-21) in the range of 1781-1683  $\text{cm}^{-1}$  (Figure 2).

### Determination of the Maximum Absorption of Ibuprofen and Andrographolide

Each 10 mg of ibuprofen and andrographolide was dissolved separately in analytical grade ethanol (for ibuprofen) and methanol (for andrographolide) to obtain 25 mcg/mL. The solutions were scanned (Figure 3) in a UV spectrophotometer (Specord 200, Analytik Jena).

### Optimization of the HPLC System

20 mcg/mL of andrographolide solution was injected into a C-18 column with a mixture

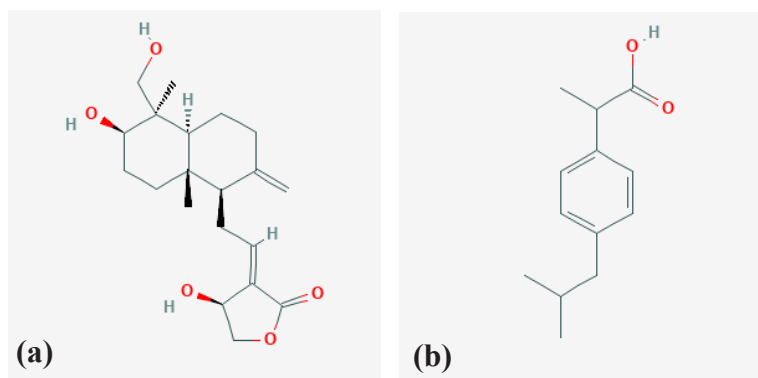


Figure 1. 2D structure of (a) andrographolide and (b) ibuprofen

of methanol: double distilled water (55:45, 65:35, and 70:30) as the mobile phase. The flow rate was 1 mL/minute and detection was set at 227 nm. Parameters observed were resolution, time of retention, and tailing factor.

#### Validation of Bioanalytical Method

Validation was performed by following the method proposed by Levita et al., 2013.<sup>12</sup>

#### Preparation of *Andrographis paniculata* Infusion

The preparation of *Andrographis paniculata* infusion was carried out by following the method proposed by Levita and co-workers.<sup>12</sup>

#### Animals

This research has been approved by The Ethics Research Committee of Universitas Padjadjaran (Document No. 1047/UN6/C1.3.2/KEP/PN/2016). Nine healthy New Zealand rabbits (6 males and 3 females, weight 1.68-2.42 kg) were purchased from Peternakan, Budidaya, dan Wirausaha Rajawali Farm. The rabbits were randomly divided into 3 groups and were acclimatized for 7 days. Standard pellet feeds (@ 75 g/ rabbit) were given twice at 08.00 and 16.00 daily.

#### The pharmacokinetic DDIs of *Andrographis paniculata* infusion and ibuprofen in the

#### plasma of healthy *Oryctolagus cuniculus* rabbits

At day-8th the rabbits were observed for their heart rate (HR), respiration rate, body temperature, and body weight at day-8th (Table 1). At the same day, the rabbits were hematology-analyzed (Table 1) and were treated with: group (1) a single oral administration of ibuprofen (dose of 28 mg/kg BW); group (2) a single oral administration of *Andrographis paniculata* infusion (7.04 mL/kg BW); group (3) a single oral administration of a mixture of *Andrographis paniculata* (7.04 mL/kg BW) infusion and ibuprofen (dose of 28 mg/kg BW). Plasma samples were prepared by collecting the blood from the marginal ear vein (Figure 5) at 0, 30, 60, 90, and 120 minutes after the mixture administration, followed by centrifuging it for 30 minutes 3000 rpm. Chromatographic separation was performed on a LiChrosorb RP-18 with methanol and double-distilled water (70:30) as the mobile phase, flow rate 1 mL/minute. UV detection was set at 227 nm.

## Results and Discussion

#### Infrared and Ultraviolet Spectrophotometry

The ultraviolet spectrum of andrographolide showed one maximum at 227 nm, which is caused by the  $\pi \rightarrow \pi^*$  transition of two double bonds (C8-C9 and C11-C12). The peak of ibuprofen occurs at 225 nm due to the aromatic transition.

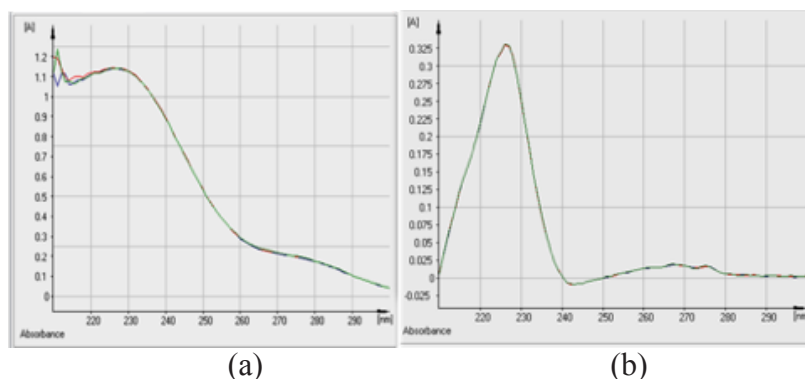


Figure 2. The UV spectrum of (a) andrographolide and (b) ibuprofen

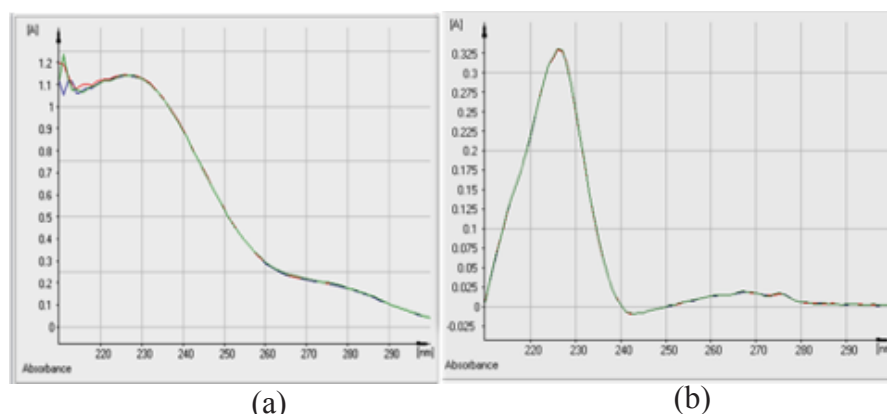


Figure 3. The infrared spectrum of (a) andrographolide and (b) ibuprofen

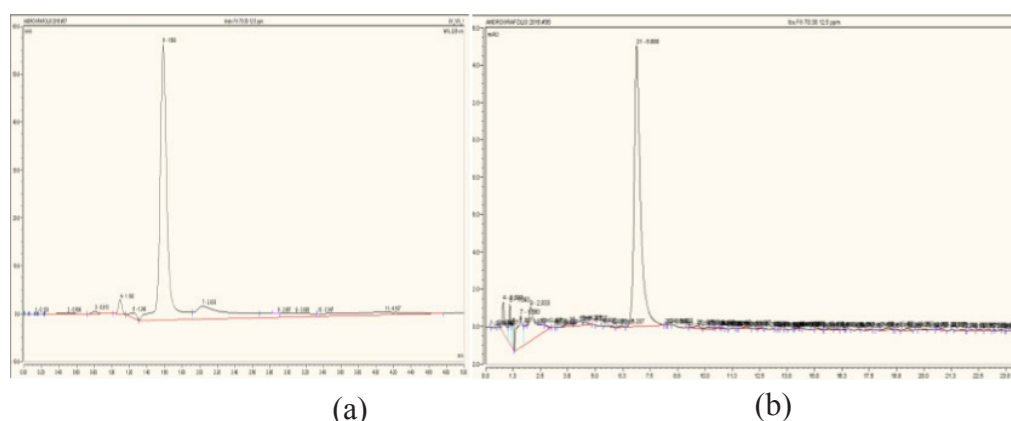


Figure 4. The HPLC chromatogram of (a) andrographolide (pure compound) and (b) ibuprofen

The IR spectrum of andrographolide (Figure 3a) reveals the presence of O-H ( $3398\text{ cm}^{-1}$ ), alkyls ( $2969\text{ cm}^{-1}$ ,  $2929\text{ cm}^{-1}$  and  $2847.92\text{ cm}^{-1}$ ), carbonyl ( $1726\text{ cm}^{-1}$ ), alkene  $\text{-C=C-}$  ( $1674\text{ cm}^{-1}$ ), while ibuprofen (Figure 3b) shows aromatics ( $1438.10\text{ cm}^{-1}$ ), carbonyl of carboxylic acid ( $1714.75\text{ cm}^{-1}$ ), and overlapped O-H with alkyl ( $2940.53\text{ cm}^{-1}$ ).

#### Optimization of the HPLC System

The optimization of the HPLC system indicated that methanol: double distilled water (70:30) is the best condition (resolution 1.39; retention factor 0.94; tailing factor 1.14; retention time 1.58 minutes). The chromatogram of andrographolide (Figure 4a) indicates a distinct peak at 1.58 minutes, while ibuprofen elutes at 6.88 minutes.

#### Validation of Bioanalytical Method

Validation of the bioanalytical method resulted in a good linearity ( $y = 0.5349x + 0.9888$ ;  $R^2 = 0.9971$ ), % recovery 93.85-107.53%; RSD = 3.976%; LLOD =  $0.11304\text{ mcg/mL}$  and LLOQ =  $0.376799\text{ mcg/mL}$ .

#### The pharmacokinetic DDIs of *Andrographis paniculata* infusion and ibuprofen in the plasma of healthy *Oryctolagus cuniculus* rabbits

During 7 days of acclimatization, all rabbits were in healthy condition as proven by their physiological status (Table 1) and biochemical parameters (Table 2).

Rabbits are defined as suffering an abnormal condition when its heart rate exceeds 300

**Table 1. Health condition of the rabbits at day-8th**

Rabbit	BW (kg)	Heart rate per minute		Respiration rate per minute		Body temperature (°C)	
		Experiment	Reference	Experiment	Reference	Experiment	Reference
1	1.80	131		60		37.33	
2	2.08	124		55		38.44	
3	2.04	147		68		38.01	
4	1.96	147		58		38.29	
5	1.68	122	130-300	64	30-60	37.76	38-40
6	2.20	139		62		38.71	
7	2.40	129		65		38.46	
8	2.42	134		62		38.41	
9	2.08	145		62		38.34	

Reference is cited from River et al<sup>15</sup>

rate/minute and respiration rate is above 220/minute.<sup>15</sup>

According to Melillo (2007) and Özkan (2012), the normal value of leucocyte and hematocrit ranges 33.10-10.57 %.<sup>16,17</sup>

The blood sampling from the marginal ear vein of the rabbit was carried out as described by Parasuraman et al. (2010)<sup>14</sup> (Figure 5).

The pharmacokinetic profile of *Andrographis paniculata* infusion (determined as andrographolide) in the plasma of healthy *Oryctolagus cuniculus* rabbits compared to those of ibuprofen alone and the mixture of ibuprofen and *Andrographis paniculata* infusion is presented in Figure 6.

Figure 6 showed that *Andrographis paniculata* (measured as andrographolide) was quantified in the plasma at 90 minutes (Figure 6: red curve), while ibuprofen was absorbed faster and reached its maximum at 60 minutes (Figure 6: blue curve; T<sub>max</sub> = 30 min; C<sub>max</sub> = 4.02962 mcg/mL). Interestingly, the absorption of *Andrographis paniculata* is enhanced by

ibuprofen as proven by its occurrence in the rabbit's blood at 60 minutes (Figure 6: green curve). These data confirmed that there is a pharmacokinetic DDIs between *Andrographis paniculata* (measured as andrographolide) and ibuprofen when these compounds are taken together at the same time.

### Conclusion

A pharmacokinetic DDIs between *Andrographis paniculata* (measured as andrographolide) and ibuprofen was observed in the plasma of healthy rabbits. Interestingly, our data indicated that the oral administration of ibuprofen together with *Andrographis paniculata* could improve the absorption and distribution of the latter in *Oryctolagus cuniculus* rabbits.

### Acknowledgement

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**Table 2. Health condition of the rabbits at day-8th**

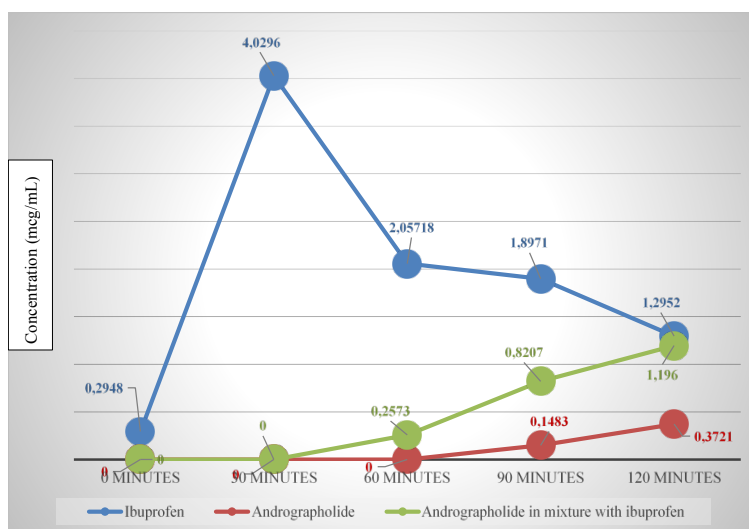
Parameter	Rabbit Code								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
Leucocyte ( $10^3/\mu\text{l}$ )	10.76	9.03	11.91	-	5.25	15.84	8.69	6.01	7.68
Lymphocyte ( $10^3/\mu\text{l}$ )	4.02	3.45	2.67	-	2.36	9.13	1.11	2.83	5.31
Monocyte ( $10^3/\mu\text{l}$ )	1.88	1.08	2.25	-	2.09	2.64	2.27	0.59	0.78
Granulocyte ( $10^3/\mu\text{l}$ )	4.85	4.51	6.99	-	0.80	4.07	5.30	2.59	1.59
Lymphocyte (%)	37.40	38.20	22.40	-	45.00	57.60	12.80	47.10	69.20
Monocyte (%)	17.50	12.00	18.90	-	39.80	16.70	26.20	9.80	10.10
Granulocyte (%)	45.10	49.90	58.70	-	15.20	25.70	61.00	43.10	20.70
Erythrocyte ( $10^6/\mu\text{l}$ )	5.37	6.49	5.63	-	4.96	5.51	4.32	5.35	4.73
Hb (g/dl)	11.60	14.70	13.00	-	12.20	12.40	10.80	12.30	11.40
Hematocrit (%)	28.92	38.00	32.32	-	31.62	33.23	27.27	31.80	29.94
Mean corpuscular volume (fl)	54	599	57	-	64	60	63	59	63
Mean corpuscular hemoglobin (pg)	21.6	22.6	23.2	-	24.7	22.5	25.1	22.9	24.2
Mean corpuscular hemoglobin concentration (g/dl)	40.2	38.6	40.4	-	38.7	37.3	39.8	38.6	38.2
Red cell distribution width (%)	18.3	16.6	16.1	-	15.5	16.4	18.4	16.4	16.2
Platelet ( $10^3/\mu\text{l}$ )	254	417	151	-	105	342	232	195	223
Plateletcrit (%)	0.14	0.13	0.04	-	0.03	0.17	0.12	0.03	0.12
Mean platelet volume (fl)	5.6	3.2	2.7	-	3.2	5	5.3	1.6	5.2
Platelet distribution width %	33.80	33	30.2	-	29.5	33.8	33.8	28.5	32.3

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**Figure 5. Blood collection from the marginal ear vein of the rabbit**



**Figure 6. Pharmacokinetic profile of ibuprofen (blue; T<sub>max</sub> = 30 min; C<sub>max</sub> = 4.02962 mcg/mL), andrographolide (red), and the mixture of *Andrographis paniculata* and ibuprofen (green) in *Oryctolagus cuniculus* rabbit's plasma**

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