CHAPTER 4

Results and Discussions

4.1 Identifications of crude drugs

4.1.1 Macroscopic characters

The crude drugs identity were evaluated followed the monographs in Pharmacopoeia of the People's Republic of China (2005) [2]. The results were shown in Table 4.1.

Table 4.1: Macroscopic characters of crude drugs used in LWDH formula

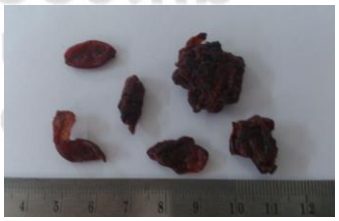
Plant name	Macroscopic characters [2]	Photos
Shudi huang	Occurance in irregular slices and pieces, broken lump, varying in size and thickness. Externally jet-black, lustrous, more sticky. Texture soft and flexible, uneasily broken, fracture jet-black, lustrous. Odour, slight; taste, sweet.	
Shan zhuyu	Irregularly flasky or bladdery, 1-1.5 cm long and 0.5-1 cm wide. Externally purplish-red to purplish-black, shrunken, lustrous. Sometimes with a rounded scar of persistent calyx at the apex and scar of fruit stalk at the base. Texture soft. Odour, slight; taste, sour, astringent and slightly bitter.	

Table 4.1: Macroscopic characters of crude drugs used in LWDH formula (continued)

Plant name	Macroscopic characters [2]	Photos
Shan yao	Cylindrical, the two ends even, 9-18 cm long, 1.5-3 cm in diameter, externally smooth, white or yellow-white.	
Zexie	Subsperical, elliptical or ovate, 2-7 cm long, 2-6 cm in diameter. Externally yellowish-white or yellowish-brown, with irregular transverse-annular shallow furrows and numerous small raised fibrous root scars, occasionally tuberculate bud scars attached to the base. Texture compact, fracture yellowish-white, starchy, with numerous small pores. Odour, slight; taste, slightly bitter.	
Fu ling	Variable in form and size. Externally brown to blackish-brown, internally white or pale brown. Relatively loose and soft, slightly elastic.	
Danpi	Quilled or semiquilled, longitudinally fissured, somewhat involute or opened, 5-20 cm long, 5-12 mm in diameter, 1-4 mm thick. Outer surface grayish-brown or yellowish-brown, showing numerous transverse lenticels-like prominences and rootlet scars, the expose surface where cork fallen off appearing pink; inner surface pale grayish-yellow or pale brown, with obvious fine longitudinal striations, usually showing bright crystals. Texture hard and fragile, easily broken, fracture relatively even, mealy pale pink. Odour, aromatic; taste, slightly bitter and astringent.	

4.1.2 Microscopic characters

The microscopic characters of 5 crude drugs (Shanzhuyu, Shanyao, Zexie, Fuling and Danpi) were evaluated followed the monographs in Pharmacopoeia of the People's Republic of China (2005) [2]. The results were shown in Table 4.2.

Table 4.2: Microscopic characters of crude drugs used in LWDH formula

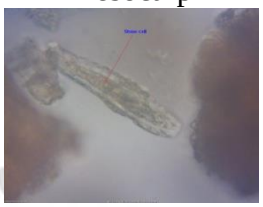
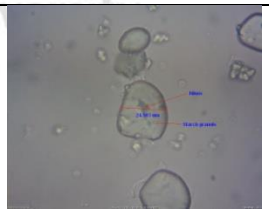
Plant name	Microscopic characters [2]	Photos
Shan zhuyu	Epidermal cell of pericarp polygonal or subrectangular in surface view, 16-30 μm in diameter, anticlinal walls beaded, outer periclinal walls granularly cutinized and thickened, lumen containing pale orange-yellow contents. Cells of mesocarp orange-brown, mostly shunken. Stone cells subsquare, ovoid or rectangular, pits obvious and with a large lumen.	 Epidermal cell  Mesocarp  Stone cell
Shan yao	Simple starch granules compressed-ovoid, deltoid-ovoid, subround or oblong, 8-35 μm in diameter, hilum point V-shape, crisscross, or shortly cleft, striations visible. Few compound starch granules, usually consisting of 2-3 granules. Mucilage cells containing raphides of calcium oxalate, up to 240 μm long and needle crystals 2-5 μm wide. Vessels border-pitted, reticulated, spiral and annular, 12-48 μm in diameter.	 Starch granule with hilum

Table 4.2: Microscopic characters of crude drugs used in LWDH formula (continued)

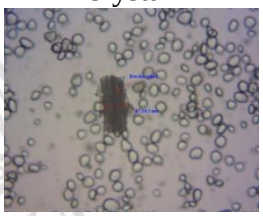
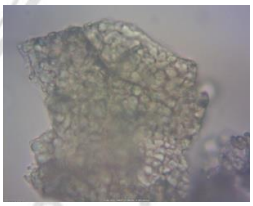
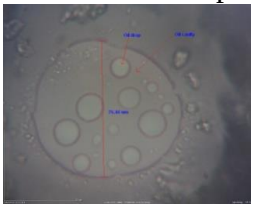
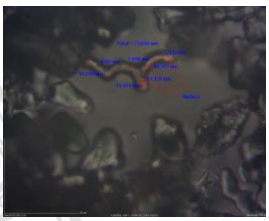
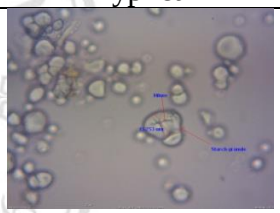
Plant name	Microscopic characters [2]	Photos
Shan yao		 Calcium oxalate crystal  Border pitted
Zexie	Starch granules numerous, simple granule long-ovoid, subsperical or ellipsoid, 3-14 μm in diameter, hilum V-shaped, shortly slit-shaped, or Y-shaped; compound granules of 2-3 components. Parachymatous cells subrounded, with many elliptical pits aggregated into pit areas. Anticlinal walls of endodermis cells sinuous, relatively thickened, lignified, with sparse and minute pit-canels, Oil cavities mostly broken, whole ones subrounded, 54-110 μm in diameter sometimes oil drops in secretory cell visible.	 Starch granule with hilum  Parenchyma cell  Endodermis with pits  Oil cavity

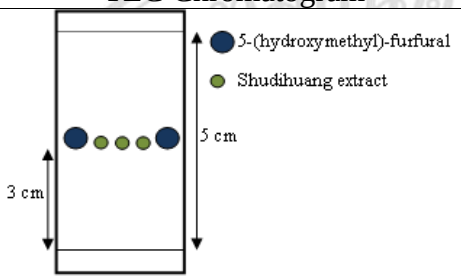
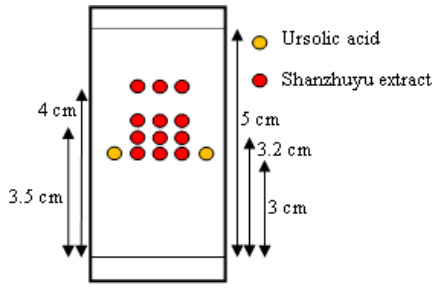
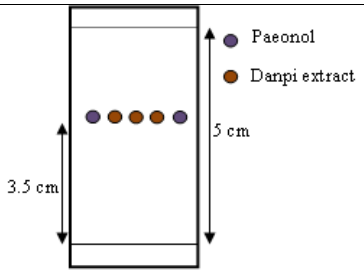
Table 4.2: Microscopic characters of crude drugs used in LWDH formula (continued)

Plant name	Microscopic characters [2]	Photos
Fu ling	Irregularly granular masses and branched mass colorless. Hyphea colorless or pale brown, slender, slightly curved; branched, 3-8 μm (rarely up to 16 μm) in diameter	 Granules  Hyphea
Danpi	Starch granules fairly abundant, simple granules subrounded or polygonal, 3-16 μm in diameter, hilum pointed, cleft or V-shaped, compound granules 2-6 components. Cluster of calcium oxalate 9-45 μm in diameter, sometimes crystal cell jointed, arrange in rows, or several clusters in one cell. Cork cell rectangular, slightly thick-walled, pale red.	 Starch granule with hilum  Calcium oxalate crystal  Cork cell

4.1.3 Chemical identifications

Shudihuang, Sanzhuyu and Danpi were tested for their chemical composition by TLC comparative to chemical marker. The results were shown in Table 4.3.

Table 4.3: TLC chromatogram of Shudihuang, Shanzhuyu and Danpi

	TLC Chromatogram	Standard Rf	Sample Rf
Shudihuang		0.6	0.6
Shanzhuyu		0.6	0.6, 0.64, 0.7, 0.8
Danpi		0.7	0.7

The test results (Table 4.1-4.2) show that both macroscopic and microscopic characteristics are in compliance with the information based on Pharmacopoeia of the People's Republic of China (2005) [2]. This result shows that the six crude drugs have appropriate required properties.

The evaluations of chemical identification for the 3 crude drugs (Shudihuang, Shanzhuyu and Danpi) were conducted by using TLC and comparing them with their standard markers. The available marker in the sample extract was determined by using retard factor (Rf) value calculation. The results (Table 4.3) showed the available marker in the extracts that gave the same Rf value as the spot of markers and confirm the correct type of crude drugs, as well.

4.2 Evaluation of crude drugs quality

4.2.1 Water content

Shanzhuyu, Fuling and Danpi were evaluated the water contents followed the method referred in the monograph. The results were shown in Table 4.4-4.5.

Table 4.4: Loss on drying of Shanzhuyu and Fuling

	Accurate weight of weighing bottle (g)	Weight of crude drug before drying (g)	After dry weight (bottle + powder) (g)	Weight of crude drug after drying (g)	Loss on drying (%w/w)	Average* (%w/w)	SD
Shanzhuyu							
Sample 1	29.23734	2.01648	31.08326	1.84592	8.46	7.99	0.55
Sample 2	29.05001	2.15678	31.03186	1.98185	8.11		
Sample 3	29.55974	2.18111	31.57970	2.01996	7.39		
Fuling							
Sample 1	28.49756	2.05949	30.42514	1.92758	6.40	6.77	0.52
Sample 2	29.51549	2.08391	31.46298	1.94749	6.55		
Sample 3	29.09524	2.33093	31.25470	2.15946	7.36		

* Upper limit of water content of Shanzhuyu and Fuling based on Pharmacopoeia of the People's Republic of China (2005) [2] is not higher than 16.0% and 15.0% respectively.

Table 4.5: Water content of Danpi

	Weight of crude drug (g)	Water volume before adding sample (ml)	Water volume after adding sample (ml)	Water volume in crude drug (ml)	Water content (%v/w)	Average *(%v/w)	SD
Sample 1	20.4354	1.7	3.2	1.5	7.34	6.75 %	0.52
Sample 2	20.4031	1.8	3.1	1.3	6.37		
Sample 3	20.6404	1.85	3.2	1.35	6.54		

*Upper limit of water content of Danpi based on Pharmacopoeia of the People's Republic of China (2005) [2] is not higher than 13.0%.

4.2.2 Total ash and acid-insoluble ash

Shudihuang, Sanzhuyu, Zexie, Fuling and Danpi were evaluated for their total ash and acid-insoluble ash. The results were shown in Table 4.6-4.7.

Table 4.6: Total ash

	Crucible accurate weight (g)	Crude drug weight before ignition (g)	weight before ignition (crucible + crude) (g)	ash weight (g)	Average (g)	Total ash* (%w/w)	SD
Shudihuang							
Sample 1	30.68895	4.59561	30.95965	0.27070	0.25457	5.55	0.015
Sample 2	32.37180	4.55070	32.61372	0.24192			
Sample 3	34.18840	4.61248	34.43950	0.25110			
Shanzhuyu							
Sample 1	32.86924	4.75224	33.09893	0.22969	0.22983	4.90	0.005
Sample 2	36.82853	4.64345	37.05333	0.22480			
Sample 3	34.79251	4.66691	35.02751	0.23500			
Zexie							
Sample 1	33.40418	4.55565	33.52804	0.12386	0.12556	2.71	0.002
Sample 2	32.42476	4.72811	32.55293	0.12817			
Sample 3	36.72388	4.60131	36.84854	0.12466			

Table 4.6: Total ash (continued)

	Crucible accurate weight (g)	Crude drug weight before ignition (g)	weight before ignition (crucible + crude) (g)	ash weight (g)	Average (g)	Total ash* (%w/w)	SD
Fuling							
Sample 1	36.22969	4.88683	36.24167	0.01198	0.01156	0.25	0.0003
Sample 2	33.74118	4.40119	33.75252	0.01134			
Sample 3	33.97156	4.80509	33.98292	0.01136			
Danpi							
Sample 1	35.70795	4.63396	35.87506	0.16711	0.16695	3.59	0.0005
Sample 2	35.34590	4.65376	35.51228	0.16638			
Sample 3	36.84984	4.67212	37.01720	0.16736			

* Upper limit of total ash of Shudihuang, Shanzhuyu, Zexie, Fuling and Danpi based on Pharmacopoeia of the People's Republic of China (2005) [2] is not higher than 6.0%, 6.0%, 5.0%, 4.0% and 5.0%, respectively.

Table 4.7: Acid-insoluble ash

	Crucible accurate weight (g)	Weight after acid adding and ignition (crucible + ash) (g)	Acid-insoluble ash (g)	Average (g)	Acid-insoluble ash* (%w/w)	SD
Shudihuang						
Sample 1	30.68895	30.77601	0.08706	0.08774	1.91	0.025
Sample 2	32.37180	32.45860	0.08680			
Sample 3	34.18840	34.27776	0.08936			
Shanzhuyu						
Sample 1	32.86924	32.88778	0.01854	0.01980	0.42	0.002
Sample 2	36.82853	36.85040	0.02187			
Sample 3	34.79251	34.81150	0.01899			
Zexie						
Sample 1	33.40418	33.40792	0.00374	0.00601	0.13	0.003
Sample 2	32.42476	32.42921	0.00445			
Sample 3	36.72388	36.73372	0.00984			
Fuling						
Sample 1	36.22969	36.23128	0.00159	0.00130	0.03	0.001
Sample 2	33.74118	33.74213	0.00194			
Sample 3	33.97156	33.97194	0.00038			

Table 4.7: Acid-insoluble ash (continued)

	Crucible accurate weight (g)	Weight after acid adding and ignition (crucible + ash) (g)	Acid-insoluble ash (g)	Average (g)	Acid-insoluble ash* (%w/w)	SD
Danpi						
Sample 1	35.70795	35.72230	0.01435	0.01299	0.28	0.001
Sample 2	35.34590	35.35863	0.01273			
Sample 3	36.84984	36.86174	0.01190			

* Upper limit of acid-insoluble ash of Shudihuang, Shanzhuyu, Zexie, Fuling and Danpi based on Pharmacopoeia of the People's Republic of China (2005) [2] is not higher than 2.0%, 0.5%, 0.5%, 2.0% and 1.0%, respectively.

4.2.3 Extractive value

Shudihuang, Shanzhuyu, and Danpi were evaluated for their extractive value by using the method referred in monographs. The results were shown in Table 4.8-4.9.

Table 4.8: Water soluble extractive value of Shudihuang and Shanzhuyu

	Crude drug weight (g)	Evaporating disk accurate weight (g)	Dry extract (20 ml) and disk weight (g)	Extract weight (20 ml) (g)	Extract value (%w/w)	Average *(%)	SD
Shudihuang							
Sample 1	4.09570	107.21360	107.78153	0.56793	69.33	69.37	0.68
Sample 2	4.17360	104.01898	104.59255	0.57357	68.71		
Sample 3	4.11062	104.58716	105.16332	0.57616	70.08		
Shanzhuyu							
Sample 1	4.00260	105.37079	105.81225	0.44146	55.15	55.07	0.30
Sample 2	4.00355	105.09208	105.53510	0.44302	55.33		
Sample 3	4.00540	108.27818	108.71666	0.43848	54.74		

* Lower limit extractive value of Shudihuang and Shanzhuyu based on Pharmacopoeia of the People's Republic of China (2005) [2] is not less than 65.0% and 50.0%, respectively.

Table 4.9: Ethanol extractive value of Danpi

Volume of ethanol adding = 50.0 ml

	Crude drug weight (g)	Evaporating disk accurate weight (g)	Dry extract (25 ml) and disk weight (g)	Extract weight (25 ml) (g)	Extract value (%)	Average *(%)	SD
Sample 1	3.05419	105.24696	105.48519	0.23823	15.60	15.63	0.36
Sample 2	2.96754	103.80557	104.03238	0.22681	15.29		
Sample 3	3.09017	108.35582	108.60301	0.24725	16.00		

* Lower limit of extractive value of Danpi based on Pharmacopoeia of the People's Republic of China (2005) [2] is not less than 15.0%.

4.2.4 Determination of chemical constituents

Shanzhuyu and Danpi were determined for their chemical marker content by using HPLC chromatograms (Figure 4.1-4.2). The results were shown in Table 4.10-4.11.

Table 4.10: Loganin content of Shanzhuyu

	Sample No.	Retention time (min)	AUC	Average AUC	RSD	Loganin content* (%w/w)
Loganin 400 µg/ml	1	6.842	14,423,552	14,412,154	0.07	[redacted]
	2	6.206	14,409,539			
	3	6.392	14,403,371			
Sample 40 mg/ml	1	6.430	10,623,653	10,601,940	0.91	0.74
	2	6.395	10,496,644			
	3	6.390	10,685,522			

* Lower limit of loganin content of Shanzhuyu based on Pharmacopoeia of the People's Republic of China (2005) [2] is not less than 0.6%.

Table 4.11: Paeonol content of Danpi

	Sample No.	Retention time (min)	AUC	Average AUC	RSD	Paeonol content* (%w/w)
Paeonol 100 µg/ml	1	43.627	5,170,974	5,173,302	0.04	
	2	43.862	5,175,177			
	3	43.839	5,173,755			
Sample 5 mg/ml	1	43.684	3,433,184	3,438,938	0.88	1.33
	2	43.718	3,412,091			
	3	43.722	3,471,538			

* Lower limit of paeonol content of Danpi based on Pharmacopoeia of the People's Republic of China (2005) [2] is not less than 1.2%.

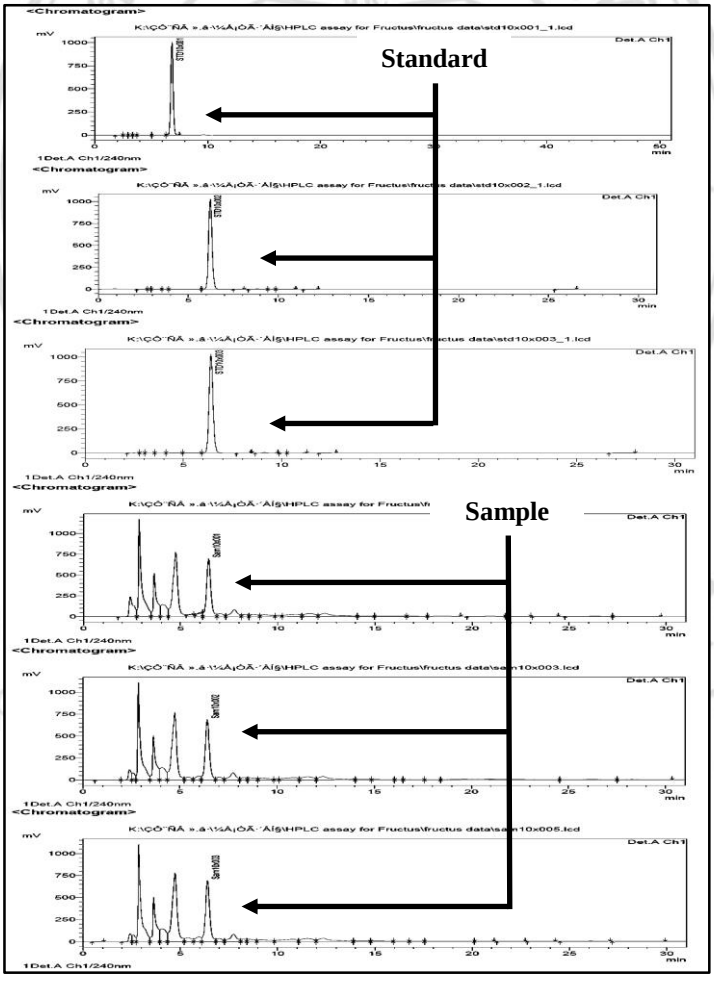


Figure 4.1: HPLC chromatograms of loganin and Shanzhuyu extract

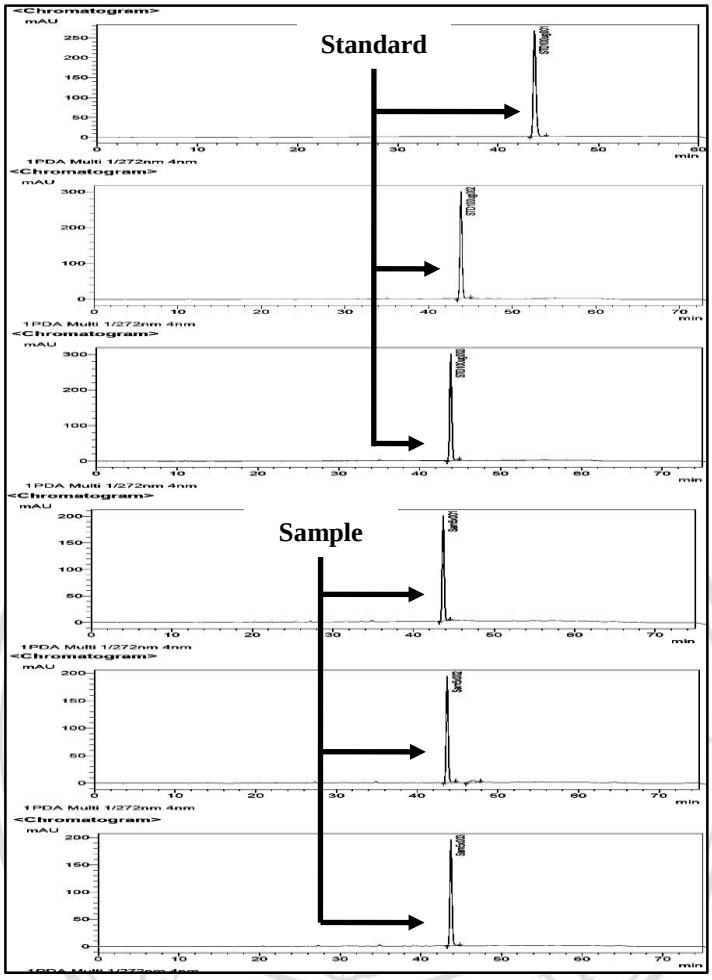


Figure 4.2: Chromatograms of paeonol and Danpi extract

Raw materials used in LWDH formula were purchased from the herbal drug stores in Thailand and then evaluated their quality followed Pharmacopoeia of the People's Republic of China (2005) [2]. Macroscopic, microscopic characters, chemical identification and other constant values of all crude drugs were accepted according to the herbal monograph (Table 4.12). These crude drugs can be used as raw materials in product development.

Table 4.12: Results of LWDH crude drugs quality

Plant name	Quality parameters				
	Water Content (%)	Total Ash (%)	Acid-insoluble Ash (%)	Extractive (%)	Marker Content (%)**
Shudihuang	n/a	5.55 (≤ 6.0)*	1.91 (≤ 2.0)*	69.37 (≥ 60.0)*	n/a
Shanzhuyu	7.99 (≤ 16.0)*	4.90 (≤ 6.0)*	0.42 (≤ 0.5)*	55.07 (≥ 50.0)*	0.74 (≥ 0.6)*
Shanyao	n/a	n/a	n/a	n/a	n/a
Zexie	n/a	2.71 (≤ 5.0)*	0.13 (≤ 0.5)*	n/a	n/a
Fuling	6.77 (≤ 15.0)*	0.25 (≤ 4.0)*	0.03 (≤ 2.0)*	n/a	n/a
Danpi	6.75 (≤ 13.0)*	3.59 (≤ 5.0)*	0.28 (≤ 1.0)*	15.63 (≥ 15.0)*	1.33 (≥ 1.2)*

n/a = No data available in the monograph
*The upper limit or lower limit of crude drugs those are available in monograph
**Standard marker: loganin for Shanzhuyu and paeonol for Danpi

4.3 Dosage determination of the water extract

The extraction of LWDH by 2 different methods; traditional decoction (TC) and reflux by water (RW) had given the yield of 55 % and 65 % (w/w) respectively. The method of extraction was different in condition control; time, temperature, therefore the extract result gave different in yielding and also the intensity of chemical composition. The quantity and quality of chemical compositions of both extracts were determined by HPLC-PDA. Loganin was selected as a chemical marker in this formula. The loganin peak was appeared in HPLC chromatogram at the retention time of 16.8 minutes. The determination was carried by calculation using AUC data from HPLC chromatograms (Figure 4.3-4.4). The results were as follows (Table 4.13).

Table 4.13: Analysis of loganin content in LWDH extract

Injection volume: loganin = 10 μl

samples = 100 μl

Sample (conc.)	Injection No.	AUC	Average	RSD	Loganin content (mg/g)	Approximate dosage of the extract
Loganin (224 μg/ml)	1	2,675,093	2,677,256	0.07		
	2	2,678,431				
	3	2,678,245				
TC (18.875 mg/ml)	1	5,580,558	5,515,420	2.30	2.44	2.0 g
	2	5,369,509				
	3	5,596,193				
RW (12.50 mg/ml)	1	5,014,786	5,109,692	1.71	3.42	1.5 g
	2	5,186,468				
	3	5,127,821				

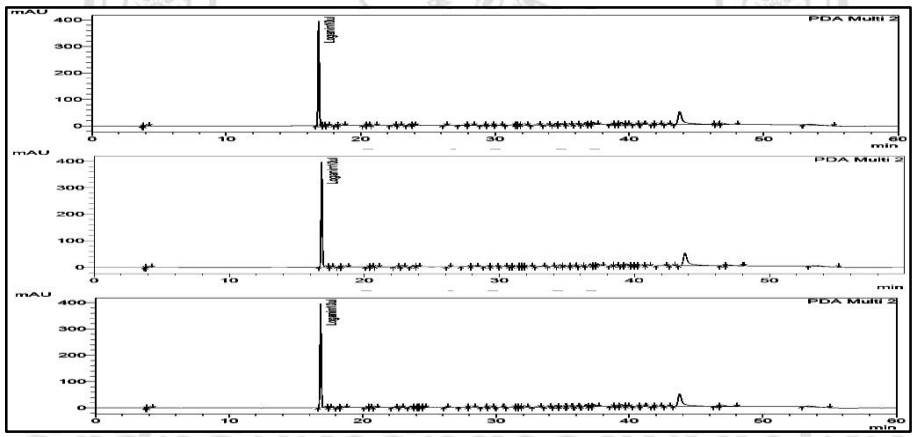


Figure 4.3: HPLC chromatograms of loganin

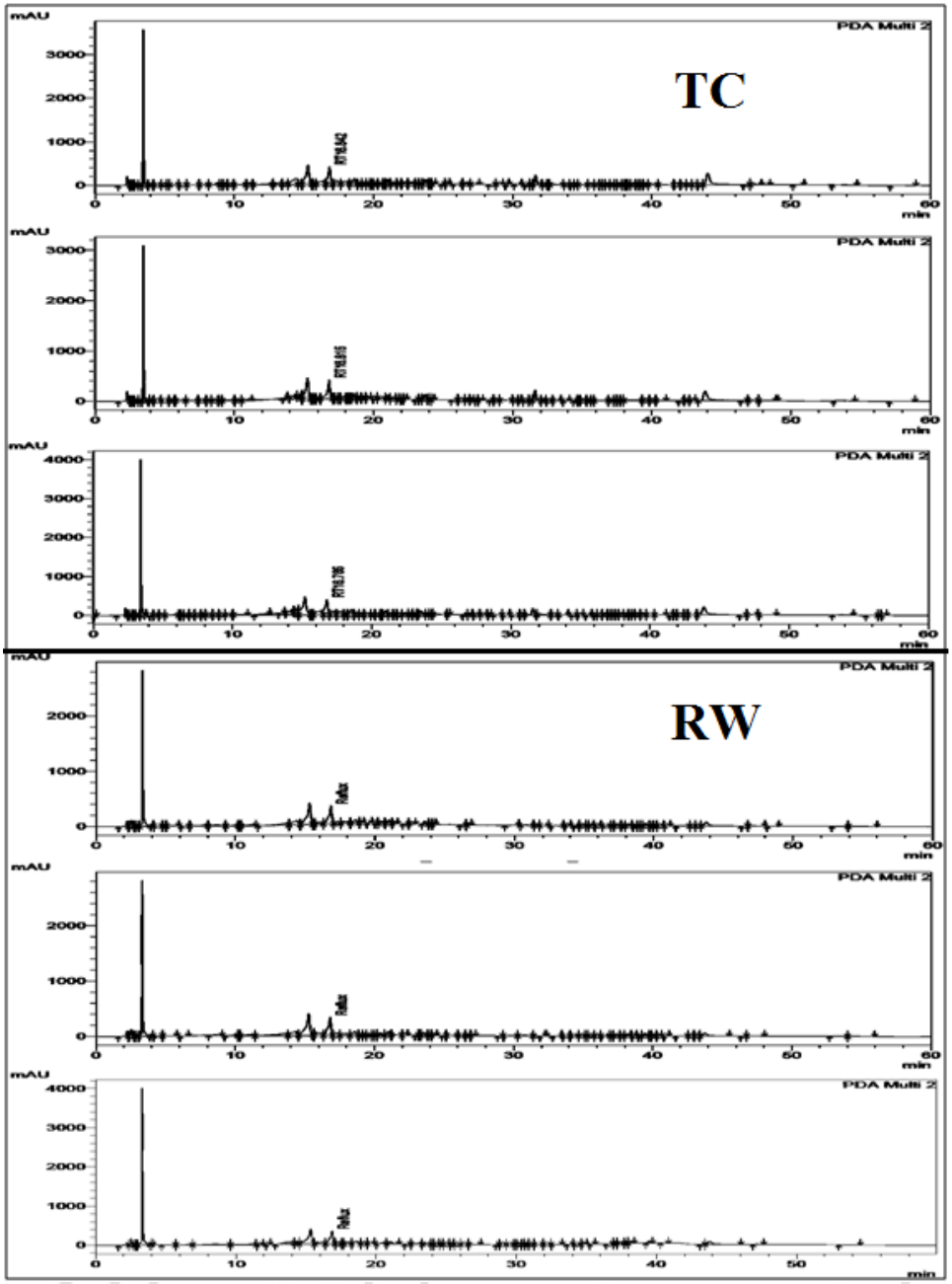


Figure 4.4: HPLC chromatograms of extract samples of TC (above) and RW (below)

In term of the ability for decoction of LWDH, 2 possible extraction methods were studied to determine the equilibrium dosage of extract. When compared with the traditional dosage form of LWDH, content of loganin must not be less than 4.5 mg/dose. According to HPLC chromatogram, the approximate dosage of RW extract

was lower than TC extract (1.5 g and 2.0 g, respectively) and also its good repeatability (RSD < 2.00) due to its better extractability and it easier endpoint observation.

In general, the typical amount of time for reflux by water extraction is at least 1 hour [52]. The endpoint of extraction by RW method in this study was 3 hours at 60-70 ^{o}C. In this study, the temperature was lower than boiling point of used solvent comparative to decoction which are boiling at the temperature of boiling point.

The drying method conducted in this study is freeze dry method which can result very high yield of the extract due to its simplify drying mechanism. However this method has a limitation of time consuming and provides energy cost. According to the limitation, the spray dry method which is a faster method to get a dried extract can be carried out. Nevertheless, there are many factors those influence the yield of the extract such as type of the atomizer, spraying pressure, inlet and outlet air temperature, etc [53]. The study about this drying method in LWDH extract should be performed in future.

Moreover, it is possible that other extraction methods, such as maceration and or continuous extraction (e.g. soxhlet's extraction) by using alcohol as solvent can decrease the dosage of extract better than RW method [54]. However, there is no data about safety and efficacy of LWDH alcoholic extract. And there is no reference in using tincture dosage form in folk wisdom.

In conclusion, RW was more suitable to be used as active ingredient in tablets dosage form than TC due to its lower dose and good repeatability.

4.4 Tablets formulation development

4.4.1 Adsorbent selection

It is normal that water extract shows the semi-solid characteristic after being evaporated as a concentrate extract. RW extract also shows this characteristic after evaporation. To develop the extract into tablets or other solid dosage form, adsorbent is one of necessary excipients [55]. Three adsorbents (lactose, corn starch and Avicel® PH101) were chosen due to water solubility (lactose [56-58]), adsorptibility (corn starch and Avicel® PH101 [56-58]) and commonly usage in Chinese herbal manufacturing [51]. The adsorbent were evaluated their suitable to be an excipient for development of tablets formula for LWDH extract by their quantity for changing the semi-solid characteristic of the extract into powder. The additional value of lactose was the highest (1:4) while the quantity of corn starch and Avicel® PH101 was intermediate and the lowest (1:2 and 1:1, respectively) (Table 4.14). In the pressure-hardness profile, the powders associated with lactose also showed their lowest hardness (Table 4.15, figure 4.5). Moreover there is a data reported that lactose is incompatible with loganin due to the glycosidic interaction between lactose and glucose unit in loganin while the other is not [51]. Thus, only corn starch and Avicel® PH101 were suitable to be excipients in LWDH extract tablets formulation development.

Table 4.14: Quantity of the adsorbents for changing the semi-solid extract into powders

Adsorbents	Extract quantity (mg)	Adding quantity (mg)	ratio
Lactose	635	2,785	1:4
Corn starch	505	1,000	1:2
Avicel® PH101	590	515	1:1

Table 4.15: Hardness of the LWDH powders associated with different type of adsorbent under compression

Adsorbents associated in powders	Hardness under compression (kg)				
	0.5 tons	1.0 tons	1.5 tons	2.0 tons	2.5 tons
Lactose	0.5	1.0	1.0	0.5	1.0
	0.5	1.0	1.0	0.5	1.0
	0.5	1.0	1.0	0,5	1.0
Corn starch	3.0	3.0	3.0	2.0	2.0
	3.0	3.0	3.0	2.0	2.0
	3.0	3.0	3.0	2.0	2.0
Avicel® PH101	5.0	5.5	6.5	6.5	7.0
	5.0	5.5	6.5	6.5	7.0
	5.0	5.5	6.5	6.5	7.0

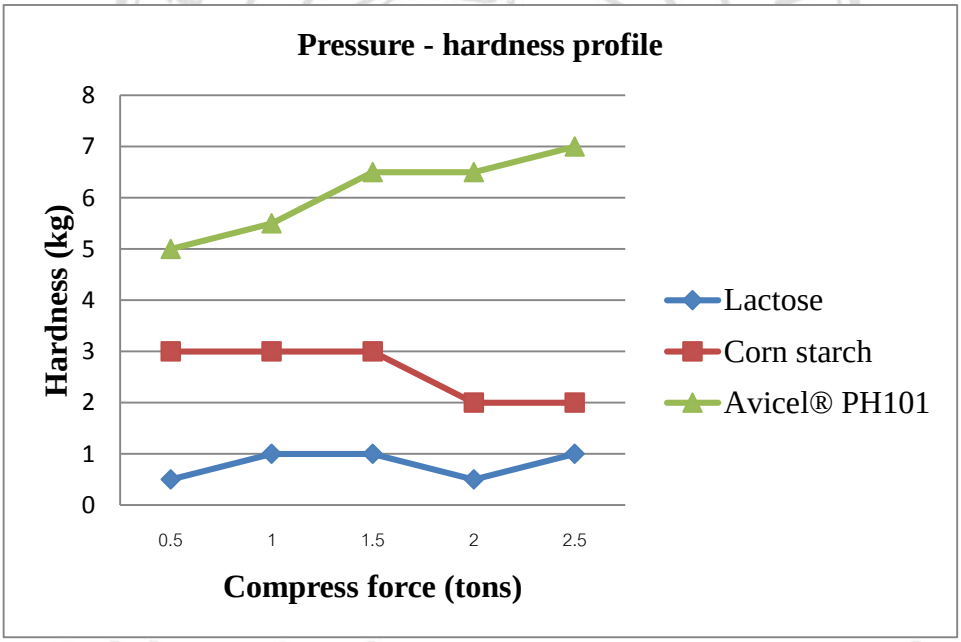


Figure 4.5: Pressure-hardness profile of the LWDH powders associated with different type of adsorbent

4.4.2 Tablets formulation development

Good tablets must be rugged to some physical stress to their easiness in carriage and also they must be disintegrated in within the required timing (30 minutes for herbal tablets) to be dissolved and absorbed in to body [35, 37, 57]. In

the development of tablets formulation of LWDH extract, 2 different batches of tablets formulation (F1 and F2) associated with adsorbents (corn starch and Avicel® PH101) were formulated with RW extract and other excipients. F1 was for the formula that corn starch was used as an adsorbent and F2 was for the formula that Avicel® PH101 was used as an adsorbent (Table 4.16). Then the batches were evaluated for preliminary study of effect of 2 different adsorbent to tablet friability and disintegration time (Table 4.17).

Table 4.16: Formulations of tablets for LWDH extract for determination of their friability and disintegration profile in the process of suitable formulation development

Ingredient	Formulations	
	F1	F2
RW extract (mg)	500	500
Avicel® PH101 (mg)	-	500
Corn starch (mg)	1000	-
Purified Talcum (mg)	15.0	10.0
Mg stearate (mg)	15.0	10.0
Cab-osil® (mg)	15.0	10.0

Table 4.17: Profile of friability and disintegration time of tablets from F1 and F2 formulation

	Friability				Disintegration	
	Weight of 20 tablets (g)		%friability	Breaking	Time (min)	Average ± SD (min)
	Before test	After test				
F1	31.6848	28.3612	10.49	8 tabs (cracking)	10.00	9.53 ± 0.72
					10.17	
					9.00	
					10.33	
					9.00	
					8.67	
F2	21.1620	21.1138	0.23	0 tab	31.17	32.00 ± 2.35
					32.00	
					30.33	
					34.50	
					29.00	
					35.00	

From the results, F1 shows to have the very good profile in disintegration (9.53 ± 0.72 min) but very poor profile in friability test (10.49%, no capping, 8 tablets cracking). On the other hand, F2 shows to be very rugged in friability (0.23%, no capping, no laminating). It can explain by the good water swelling property of corn starch that promote tablets disintegration also corn starch is not good to be binder in tablets formulation [56] thus high friability was shown in tablets of F1. In case of F2, Avicel® PH101 is one of some excipients those have both properties to be as binder and disintegrant but in higher quantity in tablets formulation (20 – 90%) it shows the property to be binder over the property to be as disintegrant which be shown in lower quantity (5 – 15%) in formulation [56].

In general, common herbal tablets are required to have the ability to disintegrate within 30 minutes [35], and to have good friability profile to reflect their physical integrity during packaging and handling [37]. Therefore, the friability and disintegration time were included in quality control parameters of common tablet from conventional medicine and herbal medicine [35, 37] (Table 2.7). Thus, the suitable formulation has to contain both corn starch (a good disintegrant) and Avicel® PH101 (a good binder) to produce the tablets that have good profile for both friability and disintegration time. Detail of components quantity associated in the suitable formulation is showed in Table 4.18.

Table 4.18: Ingredients of suitable tablets formulation of LWDH extract which was developed by using the profile of friability and disintegration in table 4.16

Ingredient	Quantity		Role
	Mg	%	
RW extract	500	38.83	Active ingredient
Avicel® PH101	375	29.13	Adsorbent/Binder
Corn starch	375	29.13	Adsorbent/Disintegrant
Purified Talcum	12.5	0.97	Anti-adherent
Mg stearate	12.5	0.97	Lubricant
Cab-osil®	12.5	0.97	Glidant

4.4.3 Quality control of LWDH tablets

The tablets of the suitable formulation were scaled up to evaluate their quality and stability. The process of development included the granule preparation (Figure 4.6). Then the granule was compressed into plain oval tablets with diameter of 23.2 x 10.3 mm and thickness of 6.7 mm, brownish-white color and tasteless (Figure 4.7). The finished tablets were studied for their quality in all aspects based on the Thai FDA principle [35]. The results are listed below (Table 4.19-4.24);

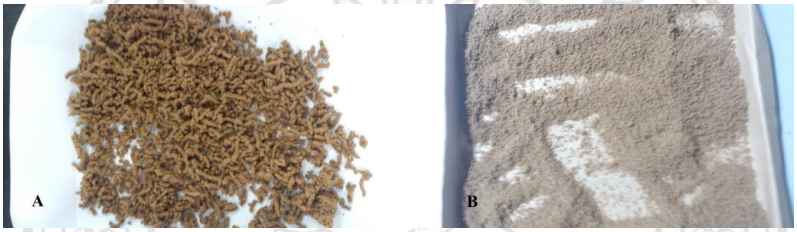


Figure 4.6: Granules before compression; wet granules (A) and dry granules (B)

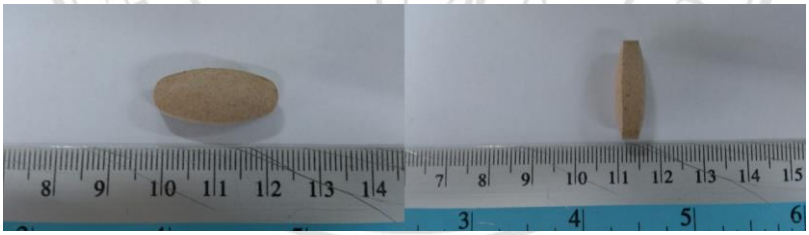


Figure 4.7: Finished plain oval tablets with diameter of 23.2 x 10.3 mm and thickness of 6.7 mm, brownish-white color and tasteless

Table 4.19: Weight variation of finished tablets

Tablet	Weight (g)	Tablet	Weight (g)	Tablet	Weight (g)	Tablet	Weight (g)
1	1.3061	6	1.3118	11	1.3266	16	1.3069
2	1.3293	7	1.3257	12	1.3062	17	1.3139
3	1.3160	8	1.3136	13	1.3163	18	1.3174
4	1.3229	9	1.3286	14	1.3056	19	1.3164
5	1.3261	10	1.3105	15	1.3263	20	1.3118
Average ± SD (variation)				1.3169 ± 0.008 g (± 0.61%)			

Table 4.20: Friability of finished tablets

	weight of 20 tablets (g)		%Friability	Amount of tablet breaking
	Before	After		
Test 1	26.3666	26.3600	0.025	0 tab
Test 2	26.3689	26.3635	0.020	0 tab
Test 3	26.3750	26.3690	0.023	0 tab
Average friability ± SD			0.023 ± 0.003%	

Table 4.21: Disintegration time of finished tablets

Tablet	Disintegration time (min)	Tablet	Disintegration time (min)
1	23.33	4	25
2	24.17	5	24.66
3	23.5	6	23.33
Average ± SD (min)		24.25 ± 0.70	

Table 4.22: Hardness of finished tablets

Tablet	Hardness (kg)	Tablet	Hardness (kg)
1	11.0	6	11.0
2	11.0	7	11.0
3	12.0	8	11.0
4	11.5	9	11.5
5	11.0	10	11.0
Average hardness ± SD		11.20 ± 0.35 kg	

Table 4.23: HPLC analysis of active marker assay of finished tablets

Sample/Standard details					
Sample	Weight of 10 tablets = 12.5549 g Average weight for 1 tablet = 1.25546 g Injection volume = 100 µl				
Standard (loganin)	Concentration = 0.224 mg/ml Injection volume = 10 µl				
HPLC results of marker content calculation					
	Injection No.	AUC	Average AUC	RSD	Loganin Content (mg/tab)
Loganin	1	2,668,874	2,667,850	0.05	
	2	2,666,250			
	3	2,668,453			
Sample	1	2,510,331	2,474,236	1.32	1.66
	2	2,465,971			
	3	2,446,407			
Desired loganin content that should have been detected for 1 tablet (mg)					1.71
%label amount of loganin in the sample					97.40%

Table 4.24: Evaluation of contamination of finished tablets

Microbial contamination			
Type	Result	Type	Result
Total bacteria	3.5 x 10^2 CFU/g	*Staphylococcus aureus*	Not detected/g
Total Fungi	< 10 CFU/g	*Salmonella* spp.	Not detected/10g
Enterobacteria	< 10/g	*Clostridium* spp.	Not detected/10g
Escherichia coli	Not detected/g		
Heavy metal contamination			
Type		Result	
Arsenic		0.1 ppm	
Cadmium		Not detected	
Lead		Not detected	

The conclusion of the results of quality evaluation of tablets for LWDH formula is showed in table 4.25. All results of quality study of the tablets, weight variation (± 0.61 from the average weight), friability (0.023 ± 0.003%), hardness (11.20 ± 0.35 kg), disintegration time (24.25 ± 0.70 min), assay of active marker (loganin content = 1.66 mg/tab = 97.40 %label amount) and contamination (lower than upper limit of Thai FDA principle in all results), are in compliance with the

specification of quality control of the Thai FDA guidelines for herbal products [35]. However, in the results of tablets disintegration time, the tablets can be disintegrated faster than the results by reduction of the tablets hardness. This may affect tablets friability but if the friability of the tablets is not more than 1%, it complied to the specification of quality control [35]. Moreover, the quantity of Mg stearate in the formulation can be reduced to get tablets with shorter disintegration time due to the usaul range of quantity of the excipient as lubricant is 0.25-2.00% [59]. However, it must be concerned about the cohesive of tablets with the tableting punch in the process of development.

Table 4.25: Conclusion of the results of quality evaluation of tablets for LWDH formula

Parameters	Guidelines criteria	Results
Appearance	Data about shape, color, odor and/or taste of the product	See figure 4.6
Weight variation	Not more than ± 15%	± 0.61%
Friability	Not more than 1%	0.023 ± 0.003%
Hardness	No data available in monograph	11.20 ± 0.35 kg
Disintegration time	Not more than 30 minutes	24.25 ± 0.70 min
Active marker (loganin) content	90 – 110 % label amount	97.40 %label amount
Contamination of microbial	- Aerobic bacteria not more than 5.0 x 10^3/g - Enterobacteria not more than 5.0 x 10^3/g - Yeast/Fungi not more than 5.0 x 10^3/g - *Escherichia coli* not more than 5.0 x 10/g - No detection of *Staphylococcus aureus* in 1 g - No detection of *Clostridium* spp. in 10 g - No detection of *Salmonella* spp. in 10 g	- Total bacteria = 3.5 x 10^2 CFU/g - Enterobacteria < 10/g - Total Fungi < 10 CFU/g - *E. coli* = Not detected/g - *S. aureus* = Not detected/g - *Clostridium* spp. = Not detected/10g - *Salmonella* spp. = Not detected/10g
Contamination of heavy metal	- Arsenic not more than 4 ppm - Cadmium not more than 0.3 ppm - Lead not more than 10 ppm	- Arsenic = 0.1 ppm - Cadmium = Not detected - Lead = Not detected

4.5 Tablets stability

The finished tablets of were packed into 2 different packaging, packaging B and packaging L (Figure 4.8), then stored in standard condition of 30ºC/65%RH and acceleration condition of 40ºC/75%RH. The stability of tablets was evaluated on day 0, day 30 and day 90. The study covered the physical stability (appearance, friability, hardness and disintegration time) and the chemical stability (Loganin content). The study results can be seen in Table 4.26. Chemical stability graphs are shown in Figure 4.9 and chromatograms are shown in Figure 4.10-4.11.

Figure 4.8: Packaging for stability evaluation of tablets; container B (left) and container L (right)

Table 4.26: Stability evaluation of tablets for LWDH extract

Parameters	Day 0	30°C/65% RH				40°C/75% RH			
		Day 30		Day 90		Day 30		Day 90	
		Container B	Container L	Container B	Container L	Container B	Container L	Container B	Container L
APP	Plain oval shape tablets with diameter of 23.2 x 10.3 mm and thickness of 6.7 mm, brownish-white color and tasteless	No significant change	No significant change	No significant change	No significant change	No significant change	No significant change	No significant change	No significant change
FRI (%)	0.023 ± 0.003	0.013 ± 0.006	0.014 ± 0.017	0.007 ± 0.004	0.010 ± 0.006	0.005 ± 0.002	0.019 ± 0.023	0.011 ± 0.006	0.009 ± 0.006
HAR (kg)	11.20 ± 0.35	11.25 ± 0.43	11.10 ± 0.39	11.10 ± 0.32	11.15 ± 0.41	11.10 ± 0.39	11.25 ± 0.43	11.05 ± 0.37	11.15 ± 0.47
DIS (min)	24.25 ± 0.70	23.83 ± 1.56	24.36 ± 0.94	23.42 ± 0.95	23.64 ± 1.10	23.70 ± 1.09	24.22 ± 0.90	24.44 ± 0.83	23.97 ± 0.93
LOG (mg/tab)	1.66	1.65	1.59	1.57	1.60	1.20	1.62	1.24	1.57

Note: APP = Appearance FRI = Friability, HAR = Hardness, DIS = Disintegration time and LOG = Loganin content

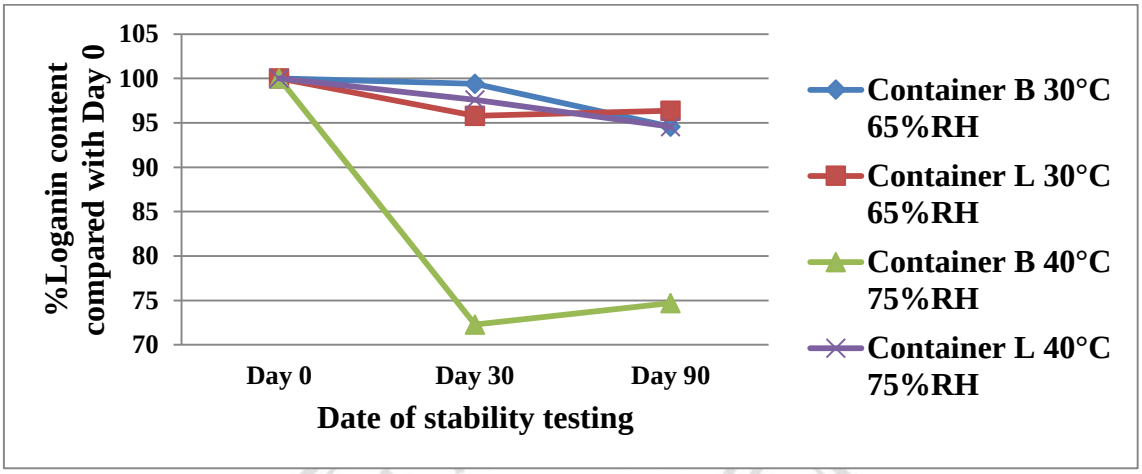


Figure 4.9: Content of loganin of tablets which were packed into 2 types different containers and stored in 2 different conditions in the period of 90 days to evaluate the product stability (lower limit = 90%)

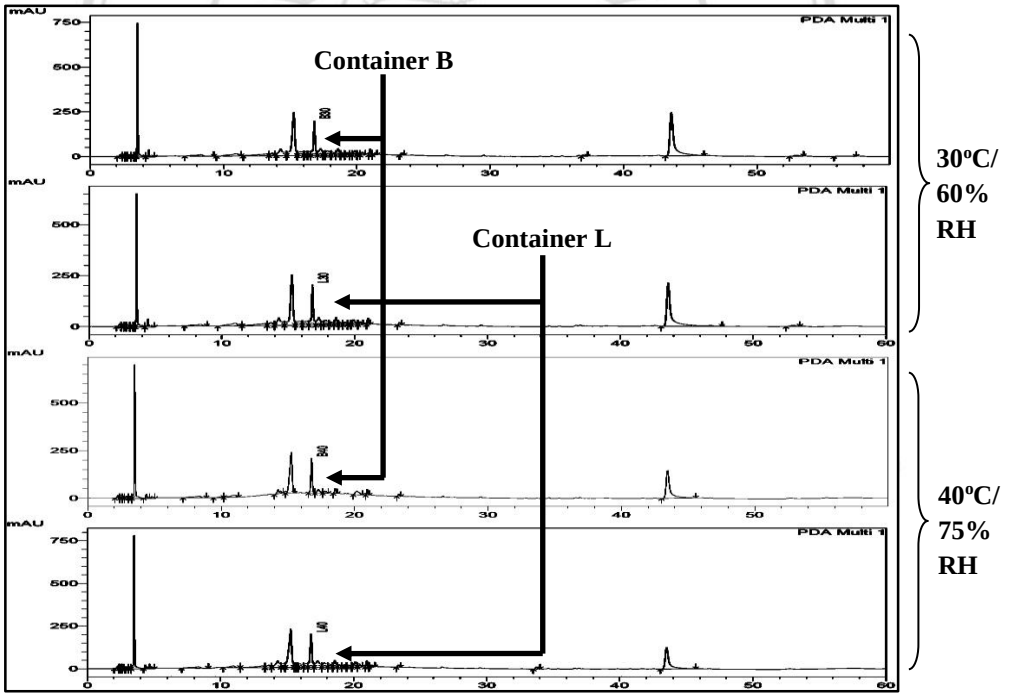


Figure 4.10: Chromatograms of samples of chemical stability testing on day 30 of 30 ºC/65 % RH and 40 ºC/75 % RH of container B and container L

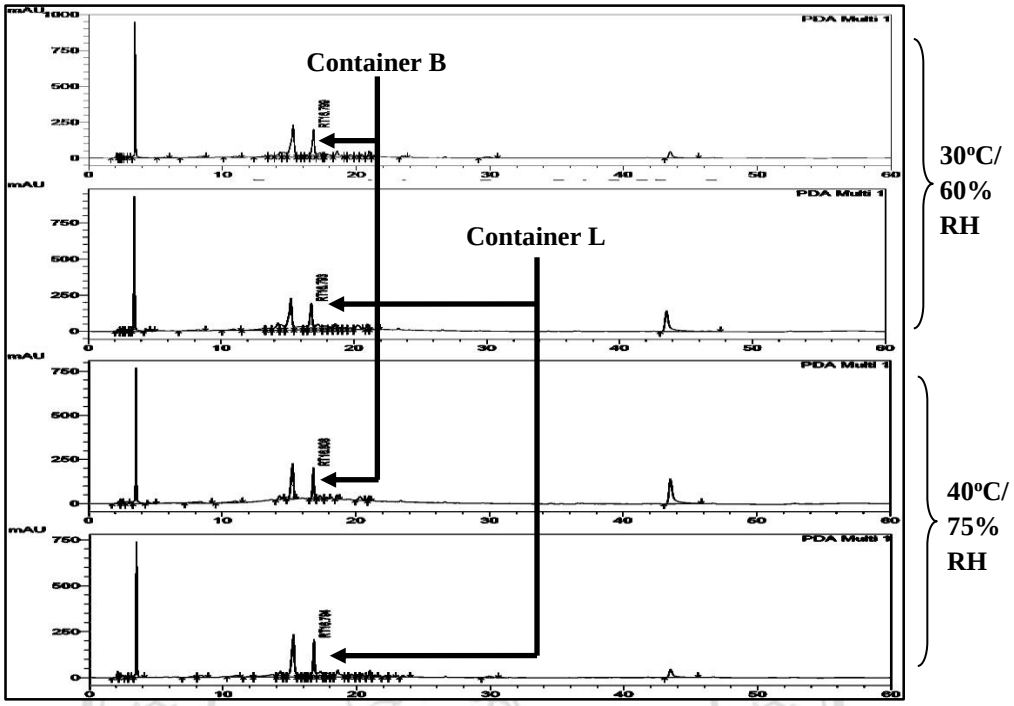


Figure 4.11: Chromatograms of samples of chemical stability testing on day 90 of 30 ºC/65 % RH and 40 ºC/75 % RH of container B and container L

From the results, the physical stability tests including the changing of appearance, friability, hardness and disintegration time, both packaging types under all conditions showed that their physical stability has met with all parameters throughout the 90-day period (Table 4.25).

For the chemical stability, both types of packaging were tested for the degradation of marker (loganin). For the standard condition of 30 ºC/60 % RH, all types of packaging show that their chemical stability of loganin content did not decrease more than 10 % from day 0. However, for the accelerated condition (40 ºC/75 % RH) tablets packed into the container B shows to have more than 10% decrease of loganin content since day 30, but those which were packed into container L showed to have loganin content over 90 % throughout the 90-day-testing (Table 4.25, Figure 4.8).

As the results, it can be concluded that moisture can affect loganin degradation and the moisture protection of the packaging is important for the prevention. Thus the

container L which has more moisture preventability than container B is the suitable container type which gives the finished tablets for to be stable over a 90 days period of the accelerated condition. Therefore, it can provide the product shelf life in container L to be more than 12 months however the long term stability evaluation must be carried out to confirm the shelf life [60].