



Analytical Method Development of Quantification and Dissolution Assay of Ursodeoxycholic Acid Capsule

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Submitted 30 December 2020; Revised 24 January 2021; Accepted 10 February 2021; Published 29 October 2021

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Abstract

Ursodeoxycholic acid is an active compound to treat cholesterol gallstones and included in Biopharmaceutical Classification System class II. Ursodeoxycholic acid capsule formulations have been developed by the pharmaceutical industries. Due to requirement, assay and dissolution tests need to be routinely carried out to control quality of the drug. Therefore, aim of this study was to develop a simultaneously analytical method for assay and dissolution test. Optimum condition of analysis were using HPLC-RID, C₁₈ column (5 µm; 3.9 x 300 mm), acetonitrile:phosphate buffer pH 3.0 (55:45 v/v) as mobile phase with flow rate 1 mL/min, 50 µL injection volume, column, detector, and sample temperature were set at 40 °C. This method provides good linearity in a range of 50-130 % concentration for assay and dissolution test with correlation coefficient values of 1 and 0.999, respectively. LOD and LOQ for assay were of 4.892 µg/mL and 14.824 µg/mL whereas for dissolution tests of 6.501 µg/mL and 19.701 µg/mL. The average percent recovery for assay and dissolution test were in a range of 100.018±0.888% and 98.936±2.124 %. Repeatability and intermediate precision of assay were obtained by determining RSD values of 0.466% and 0.279-0.483 %, while the dissolution test were 1.137% and 0.032-0.289 %.

Keywords: Assay, dissolution test, Ursodeoxycholic acid, validation

Pengembangan Metode Analisis Uji Kadar dan Uji Disolusi Kapsul Asam Ursodeoksikolat

Abstrak

Asam ursodeoksikolat merupakan senyawa aktif obat untuk mengobati batu empedu kolesterol yang termasuk dalam Biopharmaceutical Classification System kelas II. Formulasi kapsul asam ursodeoksikolat banyak dikembangkan oleh industri farmasi sehingga pengujian bahan aktif dan uji disolusi perlu dilakukan secara rutin untuk mengontrol kualitas obat. Oleh karena itu, tujuan penelitian ini adalah pengembangan metode analitis yang dapat digunakan untuk penetapan kadar dan uji disolusi secara bersamaan. Penelitian ini menggunakan KCKT dengan detektor indeks bias, kolom C₁₈ (5 µm; 3,9 x 300 mm), fase gerak asetonitril:dapar fosfat pH 3,0 (55:45 v/v) dengan laju alir 1 mL/menit, volume penyuntikan 50 µL, suhu kolom, detektor, dan sampel 40 °C. Metode ini memberikan linearitas yang baik pada rentang konsentrasi 50-130 % untuk penetapan kadar dan uji disolusi dengan nilai koefisien korelasi masing-masing 1 dan 0,999. Batas deteksi dan kuantisasi untuk penetapan kadar 4,892 µg/mL dan 14,824 µg/mL, untuk uji disolusi 6,501 µg/mL dan 19,701 µg/mL. Rata-rata persen perolehan kembali penetapan kadar dan uji disolusi berturut-turut 100,018±0.888% dan 98,936±2.124%. Keberulangan dan presisi antara penetapan kadar diperoleh nilai RSD 0,466% dan 0,279-0,483 %, sedangkan uji disolusi sebesar 1,137% dan 0,032-0,289 %.

Kata Kunci: Asam ursodeoksikolat, KCKT, penetapan kadar, uji disolusi, validasi

1. Introduction

Ursodeoxycholic acid ($C_{24}H_{40}O_4$) or UDCA is an active drug compound and widely used in capsule, tablet, and liquid formulations for the treatment of cholesterol gallstones and has recently been introduced to treat cholestatic liver disease.^{1,2} Ursodeoxycholic acid is a class II in Biopharmaceutical Classification System (BCS) with low solubility and high permeability.^{3,4} Ursodeoxycholic acid is a hydrophilic bile acid found in small amounts in humans.⁵

Ursodeoxycholic acid capsule formulations has been developed by the pharmaceutical industries. Therefore, the development of new methods and optimization of drug analysis and validation are important tasks in pharmaceutical science. According to drug regulatory agencies, the determination of the levels of active substances in drugs and dissolution testing are important factors in controlling quality of the drugs.⁶

Several studies related to the analysis of ursodeoxycholic acid in formulations have been reported, including using the HPLC-DAD with derivatization,⁷ UV-Vis spectrophotometer method with derivatization⁸, HPLC with a UV detector^{9,10,11}, and HPLC with refractive index detector.¹² From previous research for analyzed of Ursodeoxycholic acid by comparative evaluation between UV detector and refractive index detector show the conventional UV detectors are less sensitive than refractive index detector to analyze compounds with low molar absorption, such as Ursodeoxycholic acid¹³ and UDCA shows moderate absorption only in short UV wavelength region 200-210 nm, therefore quantification of the amount released from the pharmaceutical preparations by simple UV spectrophotometry was hampered by possible interferences from excipients in formulation and components in dissolution medium.¹⁴ Hence, refractive index detector could be selected because it is a universal detector since it monitors a bulk property of analyte and very suitable for analyzing compounds without a strong UV chromophore such as Ursodeoxycholic acid.¹⁵

From all previous studies, development and validation method was only focus for assay or only for dissolution test of Ursodeoxycholic acid in formulations. Therefore, in this study carried out the development and validation of the HPLC method analysis with a refractive index detector that can be used for assay and dissolution test of Ursodeoxycholic acid capsules simultaneously with the same chromatographic conditions system and also effective that never reported before. This method is expected to be used as a quality control in pharmaceutical company research laboratories for routine determination of Ursodeoxycholic acid capsule tests. The development assay method and dissolution test with the HPLC that used for drug determination were validated according to the International Conference on Harmonization (ICH) and the United State of Pharmacopoeia (USP).

2. Methods

2.1. Equipments

Analytical scales (Mettler Toledo and Sartorius), beaker glass (Pyrex), ultrasonicator bath (Branson 8800), pipettes, Whatman quantitative filter paper (Millipore), HPLC-refractive index detector (Waters 2414), filters hydrophilic 0.20 μ m (Agilent), spatula, volumetric flask (Iwaki), measuring cup (Pyrex), column L_1 (C_{18}) 5 μ m; 3.9 x 300 mm (Waters), pH meter (Mettler Toledo MP-220), volumetric pipette (Iwaki), dissolution apparatus (Vission Elite G-2 Hanson), oven (Mettler), TLC Visualizer (Camag) and vacuum filter (Millipore).

2.2. Materials

Ursodeoxycholic acid reference standard (Sichuan Xieli), Ursodeoxycholic acid capsules (PT. Kimia Farma), KOH (Merck), distilled water, sodium lauryl sulfate, KH_2PO_4 grade ISO (Merck), H_3PO_4 80% grade ACS-ISO-Reag. Ph Eur (Merck), acetonitrile HPLC grade (J.T.Baker), methanol HPLC grade (J.T.Baker), NaOH grade ISO (Merck), HCl grade ACS-ISO-Reag. Ph Eur (merck), H_2O_2 grade ISO

(Merck) dan placebo (PT. Kimia Farma).

2.3. Procedure

2.3.1. Preparation of Mobile Phase

The mobile phase was prepared from a mixture of acetonitrile and 0.075 M KH_2PO_4 solution with a ratio (55:45 v/v) and then homogenized. The pH of the solution was adjusted to 3.0 with the addition of phosphoric acid. Then the solution was filtered with a 0.2 μm filter.

2.3.2. Preparation of Dissolution Medium

Preparation of dissolution medium refers to USP 42nd by Weighed 6.8043 g of KH_2PO_4 and 3.1419 g of KOH into a 1000 mL beaker glass, dissolved in 530 mL of distilled water then added with 5 mL of 2% Sodium Lauryl Sulfate. The pH of the solution is adjusted with dilute KOH to a pH of 8.4. Furthermore, the distilled water was added to 1000 mL

2.3.3. Preparation of Standard Solution

Preparation of standard solution for assay refers to USP 42nd by weighed ± 40 mg of Ursodeoxycholic acid reference standard into a 10 mL volumetric flask. Then 5 mL of methanol was added, sonicated for 15 minutes, and methanol was added again until the limit mark (Solution A). Furthermore, 5 mL of solution A was piped into a 25 mL volumetric flask, dissolved in the mobile phase up to the limit mark, and homogenized (reference standard concentration: 800 $\mu\text{g}/\text{mL}$). Then the solution was filtered with a 0.2 μm filter.

Preparation of standard Solution for dissolution test refers to USP 42nd by weighed ± 25 mg of Ursodeoxycholic acid reference standard into a 100 mL volumetric flask, 5 mL of methanol was added and sonicated for 15 minutes. Furthermore, the dissolution medium was added to the limit mark, shaken and homogenized (reference standard concentration: 250 $\mu\text{g}/\text{mL}$). Then the solution was filtered with a 0.2 μm filter.

2.3.4. Preparation of Simulation Sample

Preparation of simulation sample

for assay refers to USP 42nd by mixed Ursodeoxycholic acid reference standard and placebo, then crushed until homogeneous. For the assay, the amount of placebo equivalent to ± 200 mg of Ursodeoxycholic acid was used, then put it in a 50 mL volumetric flask, 40 mL of methanol was added and sonicated for 15 minutes, and then methanol was added back to the limit mark (Solution A). Then 5 mL of solution A was piped into a 25 mL volumetric flask, diluted with the mobile phase up to the limit mark (simulation sample concentration: 800 $\mu\text{g}/\text{mL}$). Then the solution was filtered with a 0.2 μm filter.

Preparation of simulation sample for dissolution test refers to USP 42nd by mixed Ursodeoxycholic acid reference standard and placebo, then crushed until homogeneous. For the dissolution test, the amount of placebo was used equal to one capsule of Ursodeoxycholic acid (± 250 mg). Then the simulation sample powder was dissolved in the medium and according to the dissolution test conditions (simulation sample concentration: 250 $\mu\text{g}/\text{mL}$). Furthermore, the filtrate test results were dissolved and filtered with a 0.2 μm filter.

2.3.5. Chromatographic conditions

Chromatographic conditions were using Column L_1 (C_{18}) 5 μm ; 3.9 x 300 mm, Flow rate 1 mL/minute, injection volume 50 μL , column, detector, and sample temperature was maintained at 40 °C. While dissolution test condition refers to USP 42nd were using paddle apparatus with 75 RPM rotation, temperature 37 °C, and dissolution time for 30 minutes.

2.3.6. System Suitability Test

System suitability test was done by injected standard solution for assay and dissolution test for 6 times and the HPLC results were evaluated against the RSD area value, retention time, resolution, the number of theoretical plates, tailing factor, and the capacity factor.

2.3.7. Solution Stability Test

The stability test of the solution was carried out by injecting a standard solution and

simulation sample for assay and dissolution test at a time range of 0, 3, 6, 8, and 24 hours, with 3 replication. The analysis results are calculated based on the % deviation value of the standard solution and the simulation sample between the conditions of the time span to the initial conditions.

2.3.8. Method Validation

Specificity: the specificity of assay and dissolution test were made of placebo, reference standard, and simulation sample solution. Then several treatments were carried out such as being heated at 105 °C for 24 hours, acidified with 1.0 N HCl to pH 2.0 for 24 hours, alkalized with 1.0 N NaOH to pH 12 for 24 hours, added 10% H₂O₂ for 24 hours, and exposed to 254 nm UV light for 1 hour. Then the results of the peak chromatogram of the analyte from various treatment conditions were observed.

Linearity: the linearity of assay and dissolution test were made 7 series concentration of Ursodeoxycholic acid standard solution (50, 70, 80, 90, 100, 110, and 130%) and then made a linear regression curve. The acceptance criteria for linearity is correlation coefficient value must be ≥ 0.999 .

Accuracy: the accuracy of assay and dissolution test were carried out on the simulation sample and standard solution in the concentration range of 80, 100, 120% and each concentration was made 3 replication. Then the percentage recovery of Ursodeoxycholic acid was calculated. Accuracy parameter are accepted if percentage recovery values of these three concentration are within the range 98-102 % with RSD < 2% for assay¹⁶ and 95-105 % with RSD < 5% for dissolution test.¹⁷

Precision: the repeatability and intermediate precision of assay and dissolution test were carried out on the simulation sample solution and 100% concentration of standard solution which made 6 times. Then the level obtained of Ursodeoxycholic acid was determined. Precision criteria are accepted if RSD value < 2% for assay¹⁸ and < 5% for dissolution test.¹⁹

LOD and LOQ: LOD and LOQ of the assay and dissolution test were calculated from the linearity curve used the following equation:

$$\text{LOD} = 3,3 \times \frac{S_{y/x}}{b}; \text{LOQ} = 10 \times \frac{S_{y/x}}{b} \text{ with } S_{y/x} = \left[\frac{\sum (y_i - \bar{y})^2}{n-2} \right]^{1/2}$$

2.3.9. Application of Developed Method

Samples of Ursodeoxycholic acid capsules 250 mg was prepared for assay and dissolution test. Furthermore, the amount of Ursodeoxycholic acid in the tested sample was calculated using a validated analytical method.

3. Results

3.1. System Suitability Test

System suitability test results at Table 1 show that all parameters evaluated fit into the acceptance criteria for System Suitability Test according to USP 42nd edition and chromatogram profile show in Figure 2.

3.2. Solution Stability Test

From the results solution stability of the assay and dissolution test, the reference standard and the simulation sample solution are still stable for 24 hours because their produced a % deviation value < 2% to the initial conditions.

Tabel 1. System Suitability Test

Parameters	Assay	Dissolution Test	Acceptance Criteria (USP)
TR (minute)	5.428	5.433	
RSD area (%)	0.319	0.261	< 2%
N	5382.480	6172.011	> 2000
Tf	1.592	1.611	< 1.7
k'	26.123	26.166	> 2
Rs	6.813	2.087	> 1.5

Tabel 2. Specificity

Condition	Assay		Dissolution Test			
	T _R UDCA in standard	T _R UDCA in simulation	T _R UDCA in placebo	T _R UDCA in standard	T _R UDCA in simulation	T _R UDCA in placebo
Normal	5.375	5.373	-	5.611	5.614	-
Thermal degradation	5.349	5.346	-	5.604	5.604	-
Alkali degradation	5.336	5.345	-	5.368	5.331	-
Acid degradation	5.367	5.366	-	5.614	5.618	-
Peroxide degradation	5.374	5.378	-	5.627	5.627	-
UV light effect	5.369	5.369	-	5.616	5.620	-

3.3. Method Validation

a. Specificity

From the results of the specificity test for the assay and dissolution test at Table 2, it can be seen there is no interference from placebo (Figure 1) and peaks of the compound degradation which has the same retention time as Ursodeoxycholic acid peak, therefore this method can capture specific analyte signals for assay and dissolution test.

b. Linearity

Linearity results for assay was tested with standard Ursodeoxycholic acid in concentration range of 400.060-1040.156 µg/mL and for dissolution test in range of 125.177-325.230 µg/mL. The calibration curve, linear regression equation, and correlation coefficient value (R) for assay are

shown in Figure 2a and for dissolution test are shown in Figure 2b. The results showed good linearity with correlation coefficient value (R) 1 and 0.9995 for assay and dissolution test respectively.

c. Accuracy

From the results of accuracy at Table 3, it was obtained that the average value of the percent recovery from three concentration ranges for the assay was 100.018±0.888% with RSD value of 0.887%. While the average value of the percent recovery from three concentration ranges for dissolution test was 98.936±2.214% with RSD value of 2.146%.

d. Precision

Precision results for assay and dissolution test are shown at Table 4. The

Tabel 3. Accuracy

Level	Sample No.	Assay	Dissolution Test
		Recovery (%)	Recovery (%)
80%	1	101.184	95.992
	2	100.815	98.418
	3	100.700	96.406
100%	1	100.534	98.839
	2	100.294	97.453
	3	99.789	99.210
120%	1	98.901	101.447
	2	99.081	100.750
	3	98.869	101.913
Average RSD		100.018±0.888	98.936±2.124

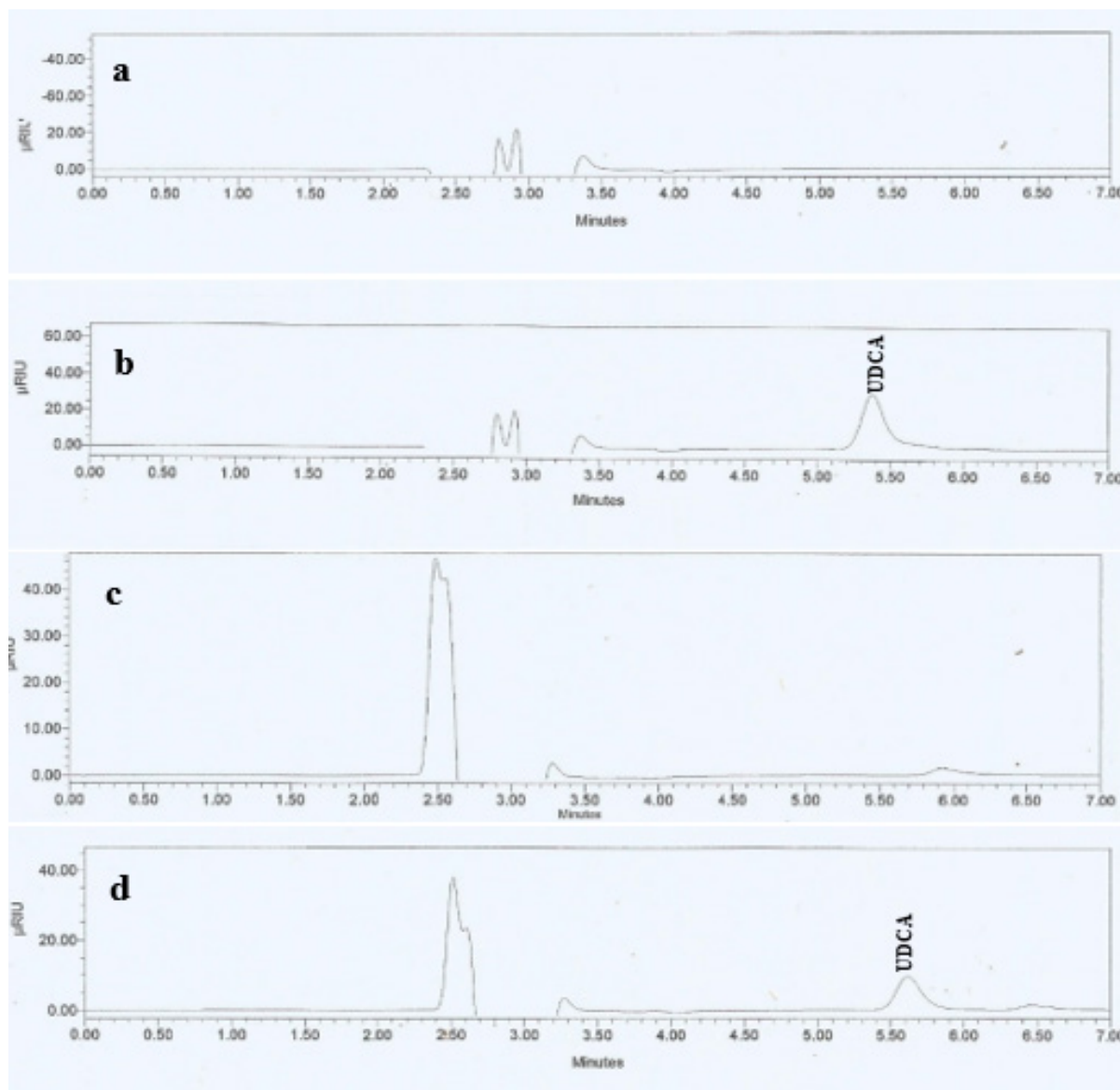


Figure 1. Chromatogram specificity of Ursodeoxycholic acid between placebo solution (a) and simulated sample solution (b) for assay; placebo solution (c) and simulated sample solution (d) for dissolution test

repeatability for assay was obtained the RSD value of 0.466% while the intermediate precision obtained the RSD value of 0.279-0.483 %. From the repeatability for dissolution test was obtained the RSD value of 1.16% while the intermediate precision obtained RSD value of 0.032-0.289 %.

e. LOD and LOQ

LOD and LOQ were calculated from the linear regression equation. LOD and LOQ for assay were 4.892 $\mu\text{g/mL}$ and 14.824 $\mu\text{g/mL}$ respectively. Whereas for the dissolution test, LOD and LOQ were 6.501 $\mu\text{g/mL}$ and 19.701 $\mu\text{g/mL}$ respectively.

3.4. Application of Developed Method

From the assay result of the Ursodeoxycholic acid capsule product, the average percent concentration obtained from 6 capsules was $103.50 \pm 1.47\%$ and the dissolved Ursodeoxycholic acid was $97.61 \pm 3.97\%$.

4. Discussion

In this research, the analytical method has been successfully developed for Ursodeoxycholic acid capsules using the HPLC-RID and the mobile phase mixture of acetonitrile:0.075 phosphate buffer pH 3.0 (55:45 v/v), so it can be used to analyze of assay and dissolution test simultaneously with

Tabel 4. Precision

No	Assay			Dissolution Test		
	Repeatability	Intermediate Precision		Repeatability	Intermediate Precision	
		Analyst 1	Analyst 2		Analyst 1	Analyst 2
		Content Obtained (mg)			Content Obtainrd (mg)	
1	201.035	200.844	197.589	247.570	246.434	244.399
2	202.827	202.641	196.804	244.116	242.366	245.991
3	200.075	200.761	197.343	248.667	246.922	245.997
4	201.016	199.678	197.202	246.412	248.195	245.539
5	201.347	201.450	198.443	246.978	248.690	245.108
6	200.552	200.925	197.643	252.566	251.492	244.497
Average	201.142	201.050	197.504	247.718	247.350	245.255
SD	0.938	0.971	0.550	2.816	0.080	0.708
RSD	0.466	0.483	0.279	1.137	0.032	0.289

*UDCA was added for assay : 200 mg; UDCA was added for dissolution test: 250 mg

the same chromatographic conditions system. The dissolution test of Ursodeoxycholic acid capsules was carried out in phosphate buffer pH 8.4 because at pH < 7 Ursodeoxycholic acid showed polymorphic transformation into water-insoluble crystals.²⁰ SLS was added to medium to increase the dissolution of poorly water soluble acidic drugs such as Ursodeoxycholic acid.²¹

System suitability test (SST) was done and used to ensure that the complete analytical system including reagents, instrument, column, and analyst are suitable for intended application.²² From the parameter results were evaluated in SST it can be concluded that analytical systems are ready to do analysis.

Solution stability test is important as a stage of prevalidation in order to estimate the time between preparation all solutions and the time when the analysis is started. From the results of solution stability test, Ursodeoxycholic acid reference standard and sample solution are stable for 24 hours in each solvent.

To assess the performance of this development method, validation parameters such as specificity, linearity, accuracy, precision, LOD and LOQ were evaluated. The validation results show that this method is specific to the analyte for assay and dissolution test because there is no interference from the compound degradation and placebo at the

peak of analyte. This result is in accordance with previous research that Ursodeoxycholic acid does not degraded when thermal, acid, alkaline, peroxide degradation, and exposed to UV light were carried out.²³

The linearity results obtained the correlation coefficient (R) for the assay and dissolution test of 1 and 0.9995, respectively. The linearity results in this study show in a fairly good R value than previous studies for assay that resulted (R) 0.9951²⁴, and (R) 0.9998²⁵. Whereas for the dissolution test from previous study the (R) value was (R) 0.9990.²⁶

The average percent recovery for assay in this study were obtained at 100.018±0.888 with RSD value of 0.887% and for dissolution test of 98.936±2.124 with RSD value of 2.146%. Based on previous research, it was found the percent recovery value for assay was 99.573% with RSD value of 0.485%.²⁷ Whereas in previous studies for the dissolution test by Sawant and Mane, the percent recovery value was 99.6% with RSD value of 0.38%. Therefore, in this study RSD value for assay was smaller than the previous research, but for the dissolution test was still greater than previous studies.

The results of repeatability and intermediate precision for assay the RSD values were 0.466% and 0.279-0.483%, respectively. From the previous research by

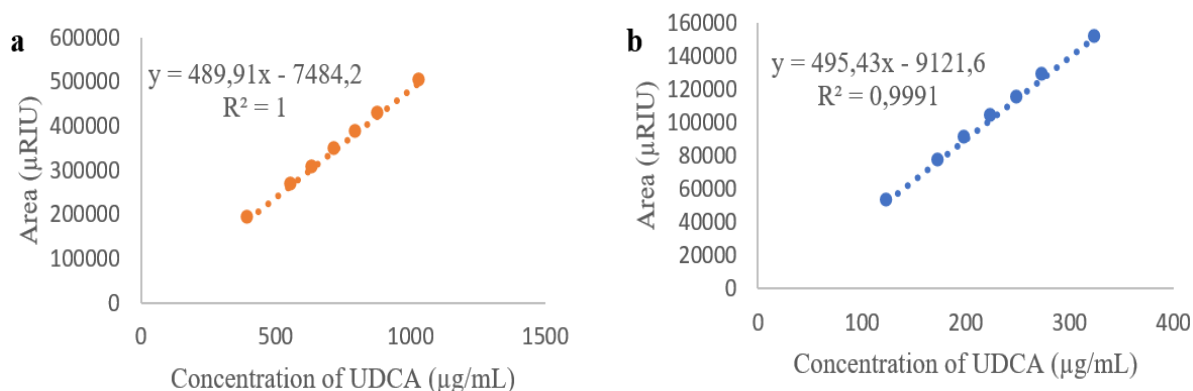


Figure 2. Calibration curve of Ursodeoxycholic acid for assay (a) and dissolution test (b)

Varinder et al., it was found the RSD value for assay was 1.94% and the intermediate precision was 1.84% therefore, this method produced a smaller RSD value compared to previous studies. Based on the repeatability and intermediate precision for dissolution test in the previous study by Sawant and Mane, the RSD values were 1.6% and 1.5% respectively. Therefore, in this study the RSD values were smaller than the previous dissolution validation studies.

The LOD and LOQ values obtained for assay were 4.892 μg/mL and 14.824 μg/mL, while the dissolution test were 6.501 μg/mL and 19.701 μg/mL respectively. While the LOD and LOQ values for assay in the previous study by El-Kafrawy et al were 8.19 μg/mL and 27.27 μg/mL. Therefore, the sensitivity of this method is higher than the previous research. Meanwhile, the dissolution validation test in the previous study did not calculate LOD and LOQ. From the LOD and LOQ values in present study, this method still satisfy for quantification of Ursodeoxycholic acid in formulation because from previous research the usual concentration of Ursodeoxycholic acid from sample formulation in the test solution in between 200-15000 μg/mL.^{7,9,10,11,14,23,24,25,26,27}

From the results assay of Ursodeoxycholic acid capsule samples, it was obtained the average concentration of 6 capsules was $103.50 \pm 1.47\%$ and Ursodeoxycholic acid dissolved was $97.61 \pm 3.97\%$. This value meets the requirements set by the USP 42nd for assay between 90-110% and dissolution test $\geq 80\%$

for Ursodeoxycholic acid capsule.

5. Conclusions

The results validation of the analytical method showed the method development in this study are linear, selective, accurate, and precise so it can be used simultaneously for the assay and dissolution test of Ursodeoxycholic acid capsule with the same chromatographic condition systems.

6. Acknowledgement

The authors would like to thank PT. Kimia Farma Plant Banjaran, Bandung, Indonesia for providing the facilities to carried out this research.

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