Improved Stability of OPALMON® Tablets under Humid Conditions IV: Effect of Polysaccharides and Disintegrants on the Stability and Dissolution Property of OPALMON® Tablets

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We studied the effects of dextran, dextrin, and disintegrants on the chemical stability of Opalmon tablets containing Limaprost-alfadex (Limaprost/α-cyclodextrin complex) and found that the addition of dextran or dextrin significantly improved the chemical stability of Opalmon tablets under high humidity, compared to lactose. We also examined how dextran stabilizes Limaprost in Opalmon tablets and studied the formulation of Opalmon tablets in order to achieve higher chemical stability, rapid dissolution and reduced stickiness. The results suggested that dextran increases stabilization after moisture adsorption by decreasing the dissociation of Limaprost-alfadex to the free drug and α-cyclodextrin in the dextran matrix, when compared with the lactose matrix. The stickiness of Opalmon tablets containing dextran and dextrin was negligible when dextran and dextrin amounted to less than 20% of the formulation. By selecting a proper disintegrant, we obtained Opalmon tablets with higher chemical stability and rapid dissolution properties.

Key words Opalmon; limaprost-alfadex; stability; formulation; dissolution

Opalmon tablet is the first oral drug containing prostaglandin E_1 (PGE_1) derivative as a pharmaceutical preparation for the circulatory system. Opalmon was approved and marketed for the adaptation disease of "confinement-related thromboangiitis ulcer, sharp pain and anaphrodisia and ischemia characteristics symptom" and "the improvement of subjective symptom and walking ability with postnatal lumbar spinal canal stenosis". Opalmon tablets contain the active pharmaceutical ingredient (API) Limaprost-alfadex, which is the inclusion complex of Limaprost, a PGE_1 derivative, with α-cyclodextrin (α-CyD). The main degradation product under high humidity is 17*S*,20-dimethyl-*trans*-Δ_2-PGA_1 (11-deoxy-Δ_{10}, Fig. 1). The aim of this study was to improve the chemical stability of unpackaged Limaprost-alfadex under high humidity. We found that the chemical stability of Limaprost-alfadex was significantly improved by adding dextran and dextrin to the tablet formulation.[1,2] To supply improved Opalmon tablets containing the freeze-dried composite of Limaprost-alfadex with dextran for moisture resistance, the new formulation must satisfy the Japanese guidelines for bioequivalence studies for formulation changes in oral drugs.[3] Furthermore, the dissolution property of the new formulation containing the freeze-dried Limaprost-alfadex and dextran has to be equivalent to that of the former lactose formulation containing freeze-dried Limaprost-alfadex and lactose. We herein report the effect of various tablet ingredients, including dextran, dextrin, lactose and some disintegrants, on the chemical stability, dissolution property, and stickiness of the tablet. Furthermore, we examined how these polysaccharides stabilize Limaprost in the tablets.

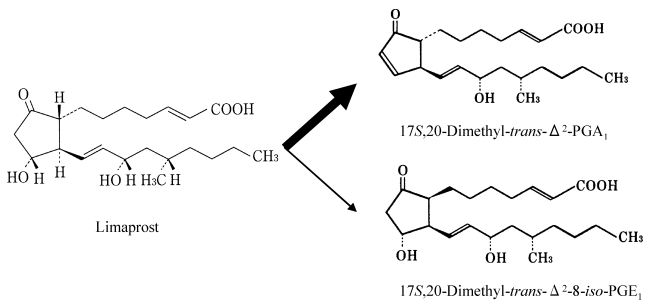

Fig. 1. Degradation Scheme of Limaprost in a Solid State

Experimental

Materials Limaprost-alfadex is specified in the Japanese Pharmaceutical Codex 2002. All excipients used were purchased with the following specifications. Lactose, dextran 40, cornstarch, dextrin, silicon dioxide and stearic acid are specified in Japanese Pharmacopoeia 15 (JP15). Calboxymethylstarch sodium is specified in the Japanese Pharmaceutical Excipients. The glucose chemical equivalence of dextrin is 8.

Opalmon Tablet Preparation To study the effect of dextran/dextrin on the chemical stability of Opalmon, tablets were prepared at different weight ratios as shown in Table 1. The freeze-dried composite (*i.e.* Limaprost-alfadex and dextran 40) was prepared as follows: Limaprost-alfadex and the appropriate excipient were dissolved in distilled water at a ratio of 1 : 7 (w/w), freeze-dried, (Triomaster, Kyowa Vacuum Engineering Ltd.) and then sieved. Dextrin, lactose and stearic acid were blended and compressed with a rotary tablet press (Virgo, Kikusui Seisakusyo Ltd.). Tablets of 100 mg/6.5 mm in diameter were prepared at 800 kg compression force. To test stability,

Table 1. Effects of Dextran and Dextrin on the Stability of Opalmon Tablets

	Form. 1	Form. 2	Form. 3	Form. 4
Lyophilized composite (1 : 7)	1.33	1.33	1.33	1.33
Dextrin	0	8.67	18.67	38.67
Lactose	97.97	89.3	79.3	59.3
Silicon dioxide	0.2	0.2	0.2	0.2
Stearic acid	0.5	0.5	0.5	0.5
Total	100	100	100	100 (mg)

Lyophilized composite (1 : 7): Limaprost-alfadex/dextran = 1/7 (w/w).

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Table 2. Effects of Disintegrants on the Stability of Opalmon Tablets

	Form. 2	Form. 5	Form. 6	Form. 7	Form. 8	Form. 9
Lyophilized composite (1 : 7)	1.33	1.33	1.33	1.33	1.33	1.33
Dextrin	8.67	8.67	8.67	8.67	8.67	8.67
Lactose	89.3	84.3	79.3	86.8	84.3	79.3
Adsolider 101	0.2	0.2	0.2	0.2	0.2	0.2
Stearic acid	0.5	0.5	0.5	0.5	0.5	0.5
Primogel	—	5	10	—	—	—
Corn starch	—	—	—	2.5	5	10
Total	100	100	100	100	100	100 (mg)

the tablets were subjected to 25 °C, 75% relative humidity (RH). To determine the effect of disintegrants on stability, tablets were prepared at the different weight ratios shown in Table 2, under the same conditions as described above and then subjected to identical conditions as describe above.

Preparation of Limaprost/α-CyD/Lactose Systems The following samples were prepared at the same molar ratio: 1) physical mixture of Limaprost-alfadex (inclusion complex with α-CyD) and lactose, 2) physical mixture of free Limaprost, α-CyD, and lactose, 3) freeze-dried compound of Limaprost-alfadex (inclusion complex with α-CyD) and lactose (freeze-dried after both are dissolved in water). The stability of these samples was tested under 48% RH and 75% RH at 40 °C. Only sample 3 was stored under 75% RH at 25 °C for 5 d before the stability test. Each experiment was repeated three times and the results are shown as mean values or mean values±standard deviation of the mean.

Assay of Free, Uncomplexed Limaprost Approximately 80 mg of powder was mixed vigorously in 2 ml of ethyl acetate solution containing the internal standard, *p*-hydroxybenzoic acid *n*-propyl ester. After centrifugation, the supernatant was fractionated, desiccated, and redissolved in 1 ml of ethanol. Free Limaprost and its degradation products, 17*S*,20-dimethyl-*trans*-Δ_2-PGA_1 and 17*S*,20-dimethyl-*trans*-Δ_2-8-iso-PGE_1, were assayed by high performance liquid chromatography (LC2010CHT, Shimadzu Corporation). Any residue remaining after centrifugation was dissolved in 3 ml of purified water and 2 ml of the internal standard solution (*p*-hydroxybenzoic acid *n*-propyl ester/ethyl acetate solution), and assayed for the Limaprost inclusion form and its degradation products as described above. The chromatographic system is described in Table 3. Each experiment was repeated three times and the results are expressed as mean values or mean values±standard deviation of the mean.

Assay for Purity & Related Substances Ten tablets were dissolved in 3 ml of purified water. Two milliliters of the internal standard solution (*p*-hydroxybenzoic acid *n*-propyl ester in ethanol solution) was added, the solution was vortexed, and 200 μl was analyzed for Limaprost and its degradation products, 17*S*,20-dimethyl-*trans*-Δ_2-PGA_1 and 17*S*,20-dimethyl-*trans*-Δ_2-8-iso-PGE_1, by HPLC (LC2010CHT, Shimadzu Corporation). The ratio of 17*S*,20-dimethyl-*trans*-Δ_2-PGA_1 and its related substances, 17*S*,20-dimethyl-*trans*-Δ_2-PGA_1 and 17*S*,20-dimethyl-*trans*-Δ_2-8-iso-PGE_1, to total Limaprost was calculated. The chromatographic system is described in Table 3. Each experiment was repeated three times and the results are reported as mean values or mean values±standard deviation of the mean.

Observation of Tablet Stickiness Tablets were placed in an open glass bottle and stored under 60% and 75% RH (25 °C), 75% RH (30 °C) or 75% RH (40 °C). The degree of stickiness between tablets and the bottom of the bottle were macroscopically observed 3 and 10 d after storage, by overturning and tapping the bottle lightly. The presence and absence of stickiness are denoted as × and ○, respectively in Table 4.

Dissolution Test The dissolution test of tablets was performed in accordance with the JP15 dissolution test using 900 ml of purified water as the dissolution medium and apparatus 2 (paddle method, UDT-804, Logan instruments Corp.) with the paddle driven at 50 rpm. Ten milliliters of the dissolution medium was collected at 10, 15, and 30 min, centrifuged, and subjected to HPLC to determine the amounts of dissolved Limaprost. Each experiment was repeated three times and the results are expressed as mean values or mean values±standard deviation of the mean. The dissolution properties of the dextran formulation and the lactose formulation were studied in accordance with the bioequivalence study guidelines.[3] Since Opalmon tablet is listed as a "product containing acidic drug" in the guidelines, the dissolution tests were performed at a paddle speed of 50 rpm using pH 1.2, 6.5, 7.5 buffer and purified water and at 100 rpm using pH 6.5 buffer. Based on the results of 12 tablets in each test condition, the dissolution properties of the test tablet and reference tablet were considered equivalent when the average dissolution of the reference product reached 85% within 15 min and the following requirements were satisfied: (1) the average dissolution of the test product also reached 85% within 15 min or did not deviate by more than ±10% from that of the reference product at 15 min and (2) the dissolution of less than 1 in 12 tablets deviated by more than ±15% from the average dissolution of the test product and no tablet in 12 tablets deviated by more than ±25% from the average dissolution of the test product.

Table 3. HPLC Conditions

Detector	UV wave (215 nm)
Column	φ4.6 mm, 15 cm length, ODS Column
Column temp.	35 °C
Mobile phase	0.02 mol/l potassium dihydrogenphosphate (pH 3.0)/acetonitrile/isopropyl alcohol (9 : 4 : 2, volume ratio)
Flow rate	0.8 ml/min

Table 4. Stickiness of Opalmon Tablets under High Humidity

Condition	Day	Form. 1	Form. 2	Form. 3	Form. 4
25 °C, 60% RH	3	○	○	×	×
	10	○	○	×	×
25 °C, 75% RH	3	○	○	×	×
	10	○	○	×	×
30 °C, 75% RH	3	○	○	×	×
	10	○	○	×	×
40 °C, 75% RH	3	○	○	×	×
	10	○	○	×	×

○: no stickiness, ×: stickiness.

Results and Discussion

Effects of Dextran/Dextrin on the Chemical Stability of Opalmon Tablets We previously reported[1,2] that dextran or dextrin improve the chemical stability of Limaprost. However, on the Japanese market, dextran content is limited in formulations for oral administration. Therefore, we studied the addition of dextrin on Opalmon tablet stability. Figure 2 shows the chemical stability of Opalmon tablets under high humidity with decreasing weight ratios of dextrin in each tablet formulation. Each formulation exhibited a gradual increase in the percentage of the degradation product, 11-deoxy-Δ_{10}. It is apparent that the dextran formulation was remarkably more stable than the lactose formulation under humidity. For example, the percentage of 11-deoxy-Δ_{10} increased up to 6.9% after 7 d in the lactose formulation (Form. 1), whereas degradation in the dextran formulations (Forms. 2—4) was only 1%. Furthermore, as the weight ratio of dextrin in the formulation increased, the percentage of 11-deoxy-Δ_{10} detected under humidity decreased. The percentage of

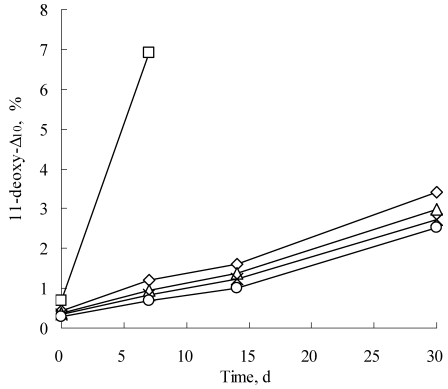

Fig. 2. Effects of Dextran/Dextrin on the Stability of Opalmon Tablets

◇: Form. 1, △: Form. 2, ×: Form. 3, ○: Form. 4, □: Former lactose formulation. Each symbol exhibits average % of 11-deoxy-Δ_{10} in each formulation.

11-deoxy-Δ_{10} was 3.4% after 30 d in Form. 1 containing no dextrin, while it was 2.9% in Form. 2 with 8.67% dextrin, 2.7% in Form. 3 with 18.67% dextrin, and 2.5% in Form. 4 with 38.67% dextrin. These data indicate that the chemical stability of Opalmon tablets under humidity is improved by freeze-drying Limaprost-alfadex and dextran and by adding polysaccharides such as dextrin to the formulation.

Effect of Dissociation of Limaprost/α-CyD Complex on Chemical Stability As we previously reported,[1] the mechanism of polysaccharide' stabilization effect on Limaprost couldn't be explained from the viewpoints of pH or water contents. And near infrared spectroscopic study and solid NMR study showed that molecular mobility of ingredients are different and that water mobility in each ingredient are different.[4] These were suggested to affect the stability of Limaprost in each formulation. In this report we studied the effect of inclusion complex formation with CyD on the stability of Limaprost. The formation mode of inclusion complex of PGs and CyDs has been already studied in detail. It was reported that the stability of PGE_1 in solid state was improved in addition of β-CyD.[5] We herein considered that the inclusion ratio of Limaprost with α-CyD could affect the stability of Limaprost in solid state .

We compared the stability of the following formulations: 1) physical mixture of Limaprost-alfadex (α-CyD inclusion complex) and lactose, 2) physical mixture of free, uncomplexed Limaprost, α-CyD, and lactose, and 3) freeze-dried composite of Limaprost-alfadex and lactose (humidified for 5 d before stabilization test).

As shown in Fig. 3, the degradation rate of Limaprost in Form. 2 (physical mixture of free Limaprost, α-CyD and lactose) was much faster than that in the Form. 1 (physical mixture of Limaprost-alfadex and lactose) and was similar to that in the Form. 3 (freeze-dried composite of Limaprost-alfadex and lactose humidified for 5 d before stabilization test). These results suggest that free Limaprost is more susceptible to degradation than complexed Limaprost. Furthermore, in the humidified, freeze-dried composite, Limaprost dissociated from CD, producing a larger ratio of free Limaprost that led to faster degradation under humidity.

α-CyD is reported to have small internal diameter. This suggests that the alkyl-side-chain of $PGF_{2\alpha}$ or PGE_1 derivative, ONO-4819, doesn't form an inclusion complex with α-CyD.[6—8] In contrast, β-CyD is reported to have large internal

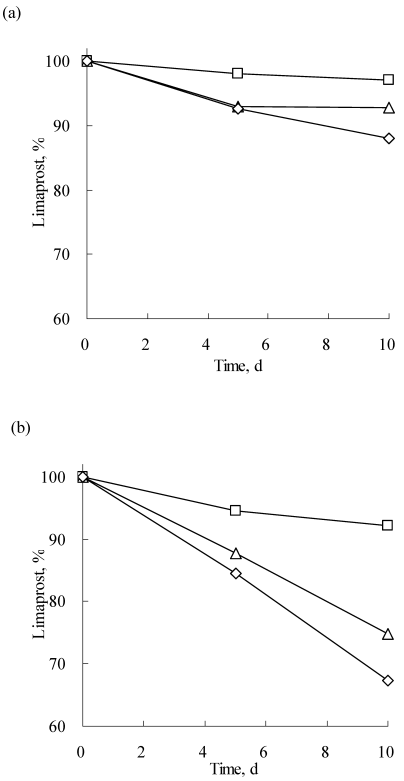

Fig. 3. Stability of Limaprost with Lactose under high humidity at 40 °C

(a) 48% RH, (b) 75% RH. □: physical mixture of Limaprost-alfadex and lactose (inclusion form), ◇: physical mixture of Limaprost, α-CyD and lactose (noninclusion form), △: freeze-dried composite of Limaprost-alfadex with lactose. Each symbol exhibits average % of Limaprost in each formulation.

diameter. This suggests that β-CyD could form an inclusion complex with the five-membered ring of PGs. Limaprost is PGE_1 derivative and it is considered that α-CyD could form a complex with its alkyl-side-chain, not five-membered ring, which is hydrolyzed under humidity. Therefore that limaprost is stabilized in inclusion state with α-CyD rather than in dissociated state could not be attributed to that the inclusion formation could protect the five-membered ring of Limaprost from contact with water molecule. Supposedly, the free Limaprost dissociated from inclusion complex in powder state became amorphous or oily and this state change could decrease the stability of Limaprost.

Therefore, we compared the contents of free Limaprost in the lactose and dextran composites stored under humidity. As shown in Fig. 4, the amount of free Limaprost in the lactose composite was about 6%, whereas it was only about 0.3% in the dextran composite, suggesting that less dissociation to free Limaprost in the dextran formulation gave superior stability. Furthermore, as shown in Fig. 5, the lactose composite melted and absorbed moisture during storage, whereas the dextran composite remained solid. Lactose is a small molecular weight compound and dissolves readily. Water-sorption of the lactose composite may allow water molecules to access the Limaprost complex, inducing dissociation to the free drug and leading to faster degradation. In contrast, dextran is a polymer and can retain its structure even after moisture-adsorption, preventing water molecules from accessing Limaprost-alfadex. This may prevent dissociation of the Limaprost complex to the free drug, leading to slower degradation. These results indicate that water-sorption followed by

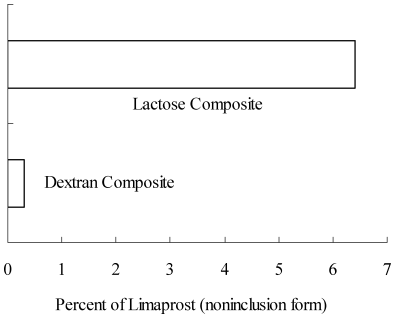

Fig. 4. Content (%) of Free Limaprost in the Lactose Composite or Dextran Composite under 40 °C, 75% RH after 10 d

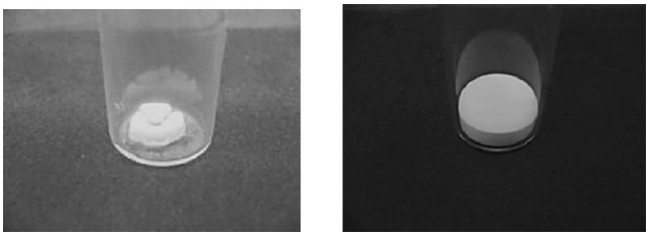

Fig. 5. Appearance of Lactose Composite (Left) and Dextran Composite (Right) under 40 °C, 75% RH after 10 d

dissociation of the complex to the free component may play an important role in the stability of Limaprost in these formulations.

Stickiness of Tablets under Humidity When large amounts of dextrin were formulated in the tablets and the tablets were stored at high humidity, they absorbed water and became sticky. Therefore, we studied the stickiness of the tablet formulations listed in Table 1. As shown in Table 4, the higher the dextrin content (>20%), the greater the tablet stickiness. These results indicate that it is better to use less than 20% dextrin in the tablet to avoid stickiness.

Effects of Disintegrants on Chemical Stability and Dissolution of Opalmon Tablets As we have already reported, cellulose excipients such as hydroxypropylcellulose deteriorate the chemical stability of Limaprost-alfadex under high humidity compared to polysaccharides such as dextran. Therefore, we studied cornstarch and starch disintegrants, such as sodium carboxymethylstarch. As shown in Fig. 6, each formulation exhibited a gradual increase in the 11-deoxy compound under humidity. In particular, the formulation containing sodium carboxymethylstarch exhibited higher degradation than the formulation containing cornstarch. Sodium carboxymethylstarch is a sodium salt that effects the surrounding pH; because the stability of Limaprost is affected by pH,[1)] sodium carboxymethylstarch likely reduces the stability of Limaprost.

Figure 7a shows the dissolution test results from formulations containing cornstarch as a disintegrant. The dissolution of Limaprost increased as the amount of cornstarch increased; the formulation containing 10% cornstarch gave more than 85% dissolution within 15 min. Figure 7b shows the dissolution test results of Forms. 2, 7, 8 and 9 before and after storage at 25 °C, 75% RH for one month. Form. 7 containing 2.5% cornstarch showed a decrease in dissolution and the dissolution rate didn't reach 85% within 15 min. In contrast, Forms. 8 and 9 containing 5% and 10% cornstarch, respectively, showed higher than 85% dissolution at 15 min. even after storage. These results show that formulations containing more than 5% cornstarch dissolve more than 85% Limaprost within 15 min with negligible reductions after storage in humid conditions. Consequently, we chose Form. 8 as a chemically stable and readily dissolvable formulation for equivalence studies of dissolution *in vitro* and absorption *in vivo* in humans given the dextran and lactose formulations. Table 5 compares dissolution between the lactose and dextran formulations. Some data in Table 5 exhibit higher than 100%. This is attributed to that the drug content is only 0.005% in each tablet. It is suggested that this significantly low drug content could lead to the analytical variability in the

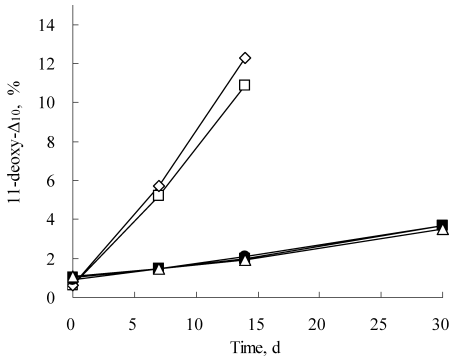

Fig. 6. The Effects of Disintegrants on the Stability of Opalmon Tablets

□: Form. 5, ◇: Form. 6, ●: Form. 7, ■: Form. 8, △: Form. 9. Each symbol exhibits average % of 11-deoxy-Δ_{10} in each formulation.

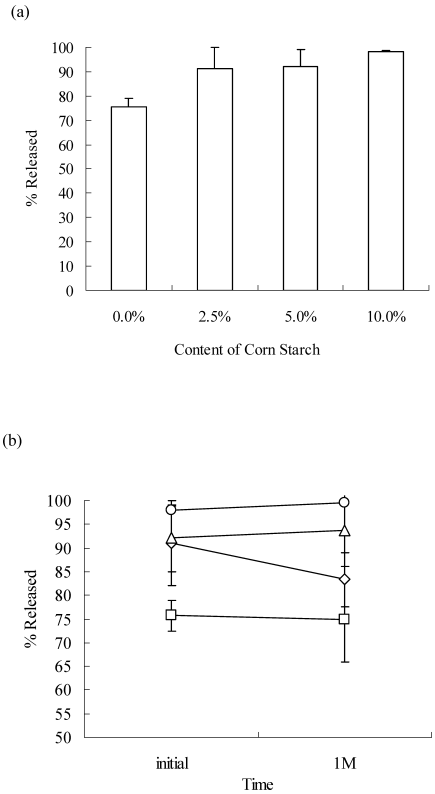

Fig. 7. The Effect of Corn Starch on the Dissolution Rate of Opalmon Tablets

(a) % of released at 15 min (initial) (b) % of released at 15 min (initial and after one month storage under 25 °C, 75% RH). □: 0%, ◇: 2.5%, △: 5%, ○: 10%.

Table 5. Opalmon Tablet Dissolution Test Results

Condition	Reference tablet (%)	Test tablet (%)
50 rpm/pH 1.2	113.7±8.0	120.7±10.5
50 rpm/water	98.1±9.6	107.2±7.2
50 rpm/pH 6.5	99.6±11.2	112.5±6.1
50 rpm/pH 7.5	92.4±11.8	111.1±6.5
100 rpm/pH 6.5	111.5±2.7	109.9±3.1

Each value exhibits dissolution rate (%) at 15 min (average±S.D.)

dissolution test. The average dissolution of the lactose formulation (reference tablets) reached 85% within 15 min. However, in the dextran formulations (test tablets), the average dissolution rate at 15 min reached 85% and didn't deviate by more than ±10% of average dissolution rate of the reference tablets. Less than one in 12 samples in each test condition (15 min) deviated by more than ±15% of the average dissolution of the test tablet, and no sample deviated by more than ±25%. We conclude that dissolution of the reference and test tablets is equivalent and that this would contribute to the bioequivalence in humans between lactose formulation and the dextran formulation.

Conclusions

In the course of studies on the effect of dextran, dextrin and disintegrants on Opalmon tablets containing Limaprost-alfadex as the API, we found that the stability of Limaprost under high humidity is significantly improved by the addition of dextran and dextrin to the formulation. The slower degradation of Limaprost in the dextran formulation was partly due to the reduced dissociation of the complex to free Limaprost that is susceptible to degradation after water-sorption. A new Opalman formulation with higher stability, rapid dissolution, and less stickiness, compared with the former formulation, was obtained by adding less than 20% dextran/dextrin and 5% cornstarch as a disintegrant in the tablet.

References

1) Sekiya N., Yamamoto M., Nishiwaki A., Nishiura A., Takeda K., *Yakuzaigaku*, **66**, 160—166 (2006).
2) Sekiya N., Abe N., Ishikawa H., Inoue Y., Yamamoto M., Takeda K., *Jpn. J. Pharm. Health Care Sci.*, **32**, 482—488 (2006).
3) "Guideline for Bioequivalence Studies for Formulation Changes of Oral Solid Dosage Forms," National Institute of Health Science, Japan, 2006.
4) Moribe K., Sekiya N., Fujito T., Yamamoto M., Higashi K., Yokohama C., Tozuka Y., Yamamoto K., *Int. J. Pharm.*, **338**, 1—6 (2007).
5) Yamamoto M., Hirayama F., Uekama K., *Chem. Pharm. Bull.*, **40**, 747—751 (1992).
6) Hirayama F., Uekama K., Koinuma H., *Chem. Pharm. Bull.*, **28**, 1975—1980 (1980).
7) Uekama K., Hirayama F., *Chem. Pharm. Bull.*, **26**, 1195—1200 (1978).
8) Uekama K., Hieda Y., Hirayama F., Arima H., Sudoh M., Yagi A., Terashima H., *Pharm. Res.*, **18**, 1578—1585 (2001).