Regular Article

Evaluation of Maltose-Induced Chemical Degradation at the Interface of Bilayer Tablets

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Fixed dose combination tablets consisting of mirabegron (MB) and solifenacin succinate (SS) were developed and formulated into bilayer tablets in the current study. The results of a chemical stability study showed that the original formulation for the tablets led to a significant increase of unknown degradants in the SS layer. Two compatibility studies were conducted to simulate the interface between the MB and SS layers, and the results revealed that the degradants only formed in the presence of both active pharmaceutical ingredients (APIs), and that the presence of maltose in the SS layer was critical to inducing degradation. High resolution mass spectroscopy coupled with high performance liquid chromatography was used to determine the chemical structures of the degradants, which were identified to MB derivatives bearing one or two sugar units. These findings therefore suggested that the degradation of the API could be attributed to the addition of sugar units from maltose to MB under the acidic conditions caused by SS. With this in mind, we developed a new formulation by replacing maltose with hydroxypropyl cellulose as a polymer-type binder. The results showed that this formulation suppressed the formation of the degradants. The results of this study have shown that chemical degradation can occur at the interface of bilayer tablets and that an alternative strategy is available to formulate more stable MB/SS bilayer tablets.

Key words bilayer tablet; interface; mass spectroscopy; maltose; compatibility

Bilayer tableting is frequently used for the formulation of fixed dose combination (FDC) tablets containing two active pharmaceutical ingredients (APIs). The fundamental structure of bilayer tablets allows for the degree of physical contact between the APIs to be minimized to avoid the potential for unwanted chemical reactivity, and this approach is therefore effective for developing stable drug products.[1–5] Despite of the advantage of bilayer tablets in development of stable formulation,[1,5] it has been reported chemical degradation could occur even in bilayer tablets.[6] Several articles have also shown that components in one of the layers of a bilayer tablet can cross-contaminate into the other layer,[7,8] leading to unfavorable quality defects (*i.e.*, chemical degradation). Based on these facts, chemical stability of bilayer tablets especially at the interface between two layers needs to be carefully and scientifically evaluated, but there are few articles which fully understood such local reaction in bilayer tablets.

Given that considerable levels of chemical degradation can occur in bilayer tablets, sensitive analytical methods are required to measure the formation of degradants in the drug product. Understanding the origin of degradants is an important part of the quality control process to keep these impurities below appropriate limits in accordance with the International Conference on Harmonisation (ICH) guideline (Q3B (R2)). However, it can be difficult to evaluate the chemical structure and origin of specific degradants using only often adopted analytical system, HPLC-UV Use of another methodology[9–11] is considered important to resolve the issue.

In this work, we have developed FDC tablets containing mirabegron (MB) and solifenacin succinate (SS) (the structures of these compounds are shown in Fig. 1). The oral dosage form currently used in clinical practice for the administration of MB is a modified release tablet which forms a hydrogel matrix consisting of polyethylene oxide and polyethylene glycol when it is contacted with water. In contrast, SS is currently formulated as an immediate release tablet for oral administration. A bilayer formulation strategy was used in the current study to develop FDC tablets consisting of MB and SS with comparable *in vitro* and *in vivo* release profiles to the corresponding mono tablets. The formulation of MB layer in

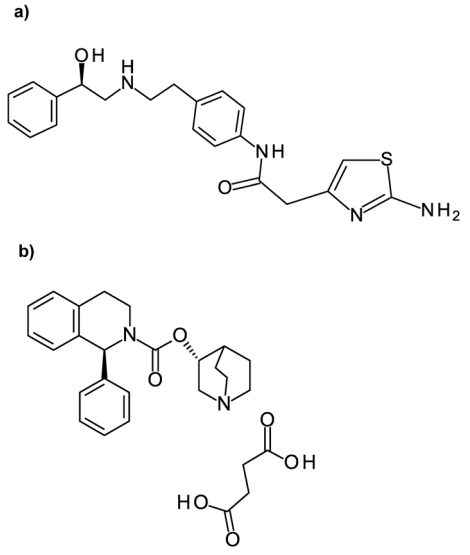

Fig. 1. Chemical Structures of (a) Mirabegron and (b) Solifenacin Succinate

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the bilayer tablets is identical to that of MB mono tablets. The formulation of SS layer was newly developed for the bilayer tablets to achieve adequate adhesion of MB and SS layers. In this study, we focused on the chemical stability of the MB/SS bilayer tablets and found several new degradants which had never been observed for the corresponding mono tablets. A series of compatibility studies were conducted to identify these degradants and elucidate the mechanism responsible for their formation. HPLC-MS analysis was also used to further confirm the chemical structures of the degradants. The results revealed that one of the excipients was responsible for the degradation of the API and allowed us to develop a new formulation strategy to overcome these chemical stability issues.

Experimental

Materials MB and SS were manufactured by Astellas Pharma Inc. (Tokyo, Japan). Polyethylene oxide was obtained from DOW Chemical (Midland, MI, U.S.A.). Polyethylene glycol was obtained from Clariant (Muttenz, Switzerland). Hydroxypropyl cellulose was purchased from Nippon Soda (Tokyo, Japan). Butylated hydroxytoluene, magnesium stearate and calcium stearate were obtained from Merck (Darmstadt, Germany). Mannitol was purchased from Roquette Freres (Lestrem, France). Maltose was obtained from Sanwa Cornstarch (Nara, Japan). Opadry® 03F43159, which was purchased as a mixture of hypromellose, talc, polyethylene glycol, titanium dioxide and ferric oxide, was obtained from Colorcon (West Point, PA, U.S.A.). All of the other materials were purchased as the analytic reagent grade and used as received.

Preparation of MB/SS Bilayer Tablets For the preparation of bilayer tablets of MB and SS, a blend of each drug layer was manufactured, followed by tablet compression and film coating. The compositions of the resulting MB/SS bilayer tablets are shown in Table 1. Two formulations containing different binding agents in the SS layer were evaluated in this study.

For the MB layer, MB was de-lumped in a screening mill with a mixture of polyethylene oxide, polyethylene glycol and hydroxypropyl cellulose. The powders were granulated in a fluid bed granulator using water as the granulating fluid. The resulting granules were blended with pulverized butylhydroxytoluene and magnesium stearate.

Table 1. Compositions of Mirabegron/Solifenacin Succinate Bilayer Tablets

Ingredients	Composition (mg/tablet)	
	Formulation *X*	Formulation *Y*
Mirabegron	25.0	25.0
Polyethylene oxide	70.0	70.0
Polyethylene glycol	144.6	144.6
Hydroxypropyl cellulose	7.5	7.5
Butylated hydroxytoluene	0.4	0.4
Magnesium stearate	2.5	2.5
Sub total	250	250
Solifenacin succinate	2.5	2.5
Mannitol	145.8	159.2
Maltose	16.7	—
Hydroxypropyl cellulose	—	3.3
Calcium stearate	1.7	1.7
Sub total	166.7	166.7
Opadry® 03F43159	12.5	12.5
Total	429.2	429.2

The SS granules were prepared using a wet granulation method. For Formulation *X*, maltose was dissolved in water, and the resulting solution was used as a binding solution for the spraying process. A blend of SS and mannitol was granulated in a fluid bed granulator. Hydroxypropyl cellulose was used instead of maltose for the preparation of the Formulation *Y*. The resulting granules were blended with calcium stearate.

The MB and SS blends described above were compressed using a bi-layered tablet compression device. The core tablets were film-coated in a perforated coating pan using the Opadry® 03F43159 solution.

MB mono tablets were prepared as a control in this study using a single MB layer by single-layer compression, followed by film-coating using the same Opadry® solution.

Evaluation of Chemical Stability of MB/SS Bilayer Tablets by HPLC-UV The MB/SS bilayer tablet samples were physically split into their MB and SS layers using a nipping device. Solid samples from each layer were ground into fine powders and extracted with methanol using an automatic shaker, followed by dilution with a mixture of water and acetonitrile (7 : 3, v/v) to final concentrations of 0.5 and 0.25 mg/mL for MB and SS, respectively.

HPLC-UV measurements were performed for the sample solutions collected from the MB and SS layers, using Waters Alliance HPLC system (Tokyo, Japan). The operating parameters for the HPLC analysis are shown in Table 2. The same mobile phases, gradient elution program, HPLC column and column temperature were used to analyze the MB and SS layers to allow for the direct comparison of the two chromatograms. The amounts of the different degradants were calculated based on a comparison of their peak areas with that of the API using the following formula:

$$\text{Amount (\%) of each degradation } (R_i) = \frac{A_i}{A_s + \sum A_i} \times 100$$

where A_s is the peak area of the API and A_i is the peak area of each degradant which is not more than 0.10% of A_s.

Compatibility Study Simulating the Interface of Bilayer Tablets Compact samples that simulate the environment around the interface between the MB and SS layers were prepared for two compatibility studies: Part 1, impact of the API; Part 2, impact of the excipient.

For Part 1, the API and excipients at the interface (all of the components except for Opadry® 03F43159) were blended in a mortar to give four combinations of the formulations, including MB (−)/SS (−), MB (−)/SS (+), MB (+)/SS (−) and MB (+)/SS (+) (Table 3). The ration by weight of MB layer to SS layer was 1 : 1 for the formulation of MB (+)/SS (+) assuming the components at the interface, and then uncontained component was just removed from MB (+)/SS (+) for the preparation of MB (−)/SS (−), MB (−)/SS (+) and MB (+)/SS (−). The blends were compacted with a single tablet press Shimadzu Autograph (Shimadzu, Kyoto, Japan) using a compression force of 10 kN with a punch of 8×16 mm (oblong). The compact samples were stressed in open vials for 4 d at 40°C/75% relative humidity (RH). Samples of these materi-

als were ground into fine powders both before and after being stored under these conditions, and extracted with methanol before being diluted with a mixture of water and acetonitrile (7 : 3, v/v) for HPLC injection. HPLC analysis was conducted using the conditions described in Table 2 for determining the degradants in the SS layer at UV 210 nm. The peak areas before and after stress storage were relatively compared among a series of the compact samples.

A separate compatibility study (Part 2) was conducted using a series of compact samples prepared with the same procedure of Part 1 study, where one or more of the excipients were excluded from the SS layer (Table 3). These samples were held for 24 h at 60°C/75% RH. HPLC analysis was performed using the same conditions as those described for Part 1.

Structural Evaluation of the Degradants by HPLC-MS The chemical structures of the degradants of the MB/SS bilayer tablets (Formulation *X*, 40°C/75% RH in open glass vials, 1 month) were analyzed by a hybrid quadrupole-orbitrap mass spectrometer (Q-Exactive, Thermo Scientific, Yokohama, Japan) coupled with a HPLC system (Nexra X2, Shimadzu, Kyoto, Japan).[12)]

An ammonium acetate buffer (pH 6.8) was used instead of a phosphate buffer (pH 6.8) because of the volatility of the former, which allowed us to conduct mass spectroscopy analysis. This replacement was only made after we confirmed that it did not have an adverse impact on the chromatographic peak profile. The HPLC system was operated under the same conditions as those described for the MB layer in Table 2, except that the injection volume was adjusted to 5 μL to obtain a desirable peak response. Furthermore, the photodiode array detector was set to a detection wavelength of 250 nm.

The MS and MS/MS data were acquired using the following parameters: capillary temperature, 250°C; S-lens level, 50.0; spray voltage, 3.0 kV; AGC target value, 2e5; sheath gas pressure, 50 arb; auxiliary air pressure, 20 arb; mass scan range, 150 to 1200 *m/z*; resolution, 140000. The positive ionization mode was applied as it showed superior response rather than the negative ionization mode.

Table 2. HPLC Conditions for the Determination of Degradants in Mirabegron and Solifenacin Succinate Layers

Parameters	Mirabegron layer		Solifenacin succinate layer
UV wavelength	250 nm		210 nm
HPLC column		XBridge® C18 150×4.6 mm i.d.×3.5 μm	
Flow rate		1.2 mL/min	
Column temperature		40°C	
Injection volume	10 μL		50 μL
Gradient program	Time (min)	% of pH 6.8 phosphate buffer	% of acetonitrile
	0→28	95→55	5→45

Table 3. Formulations of the Compact Samples for the Interface Compatibility Studies: Part 1, Impact of the API; Part 2, Impact of the Excipient

Ingredients	Formulations for Part 1 study			
	MB (−)/SS (−)	MB (−)/SS (+)	MB (+)/SS (−)	MB (+)/SS (+)
Mirabegron	−	−	+	+
Polyethylene oxide	+	+	+	+
Polyethylene glycol	+	+	+	+
Hydroxypropyl cellulose	+	+	+	+
Butylated hydroxytoluene	+	+	+	+
Magnesium stearate	+	+	+	+
Solifenacin succinate	−	+	−	+
Mannitol	+	+	+	+
Maltose	+	+	+	+
Calcium stearate	+	+	+	+

Ingredients	Formulations for Part 2 study							
	A	B	C	D	E	F	G	H
Mirabegron	+	+	+	+	+	+	+	+
Polyethylene oxide	+	+	+	+	+	−	−	−
Polyethylene glycol	+	+	+	+	+	−	−	−
Hydroxypropyl cellulose	+	+	+	+	+	−	−	−
Butylated hydroxytoluene	+	+	+	+	+	−	−	−
Magnesium stearate	+	+	+	+	+	−	−	−
Solifenacin succinate	+	+	+	+	+	+	+	+
Mannitol	+	+	+	−	−	−	−	+
Maltose	+	+	−	+	−	−	+	−
Calcium stearate	+	−	+	+	−	+	−	−

" + and –" indicate "presence and absence" of an ingredient in the compact sample, respectively.

Results and Discussion

Chemical Stability of the MB/SS Bilayer Tablets The chemical stability characteristics of the MB/SS bilayer tablets that were prepared using Formulation *X* (Table 1) were examined by HPLC-UV analysis. The tablets were subjected to humidity stress (40°C/75% RH in open glass vials) and heat stress (50°C in heat-sealed aluminum bags), as well as being subjected to long-term and accelerated storage conditions (25°C/60% RH and 40°C/75% RH) in aluminum/aluminum blisters (*i.e.*, the packaging materials used for commercial MB mono tablets in several countries). MB mono tablets were also evaluated as a control.

Tables 4 and 5 summarize the degradant results obtained for MB mono tablets and for each drug layer of the MB/SS bilayer tablets, respectively. The data includes impurities initially contained (*i.e.*, impurities and degradants from drug substance or tablet manufacturing) and degradants increasing during storage. The MB mono tablets showed good chemical stability under the long-term storage condition in the aluminum/aluminum blister packaging. The levels of some of the degradants increased when the tablets were stored under the heat or humidity stress conditions. However, their amounts were well-controlled under the long-term storage condition within identification limit (0.2%) specified in ICH guideline (Q3B (R2)). In the MB layer of the bilayer tablets, two additional degradants were increased considerably under the two stress conditions tested in the current study. A comparison with the results for the MB mono tablets revealed that MB exhibited a much poorer chemical stability in the bilayer tablets. The degradation of MB was induced to a greater extent under the humidity stress condition (40°C/75% RH in open glass vials) compared with the heat stress condition (50°C in sealed aluminum bag). Regarding chemical stability of SS layer, since a few degradants exceeding identification limit (0.2%) of ICH guideline (Q3B (R2)) were observed in the accelerated stability testing, the bilayer tablet is unlikely to pass long-term stability target when continue storage up to a couple of years. Considerable levels of degradation were also observed in the SS layer of the bilayer tablets under the stress and accelerated storage conditions. Furthermore, the degradation of the

Table 4. Chemical Stability Data of Mirabegron Mono Tablets

			Stability condition				
				Aluminum/aluminum blister		Sealed aluminum bag	Open glass vial
			Initial	25°C/60% RH (long-term)		50°C (stress)	40°C/75% RH (stress)
				12 months	24 months	1 month	1 month
% Degradant (retention time)	MB mono tablets	Lot A	0.11% (18.4 min)	0.13% (18.4 min)	0.13% (18.4 min)	Not tested	Not tested
		Lot B	0.15% (18.4 min)	Not tested	Not tested	0.26% (18.4 min)	0.28% (18.4 min)
			—			—	0.23% (25.2 min)

Degradant ≥0.10% is described (hyphen indicates <0.10%). Measurement was performed at $n=1$.

Table 5. Chemical Stability Data of Mirabegron/Solifenacin Succinate Bilayer Tablets with Formulation *X*

		Stability condition					
			Aluminum/aluminum blister			Sealed aluminum bag	Open glass vial
		Initial	25°C/60% RH (long-term)	40°C/75% RH (accelerated)		50°C (stress)	40°C/75% RH (stress)
			6 months	3 months	6 months	1 month	1 month
% Degradant (retention time)	MB layer	—	—	—	—	—	0.23% (16.1 min)
		—	—	—	—	—	0.23% (16.9 min)
		0.10% (18.4 min)	0.11% (18.4 min)	0.15% (18.4 min)	0.18% (18.4 min)	0.19% (18.4 min)	0.18% (18.4 min)
		—	—	—	—	0.13% (25.2 min)	0.16% (25.2 min)
	SS layer	—	—	—	—	—	0.11% (10.3 min)
		—	—	—	0.12% (10.9 min)	0.23% (10.9 min)	0.98% (10.9 min)
		—	—	—	—	0.16% (11.2 min)	0.57% (11.2 min)
		0.17% (16.1 min)	0.17% (16.1 min)	0.10% (16.1 min)	—	0.25% (16.1 min)	0.30% (16.1 min)
		—	—	—	—	—	0.21% (16.5 min)
		—	0.14% (16.9 min)	0.42% (16.9 min)	0.42% (16.9 min)	1.33% (16.9 min)	2.07% (16.9 min)
		—	—	—	0.14% (17.8 min)	0.29% (17.8 min)	0.79% (17.8 min)
		—	—	—	—	—	0.32% (18.5 min)
		—	—	0.10% (20.9 min)	—	—	0.10% (20.9 min)
		0.20% (23.0 min)	0.19% (23.0 min)	0.23% (23.0 min)	0.32% (23.0 min)	0.29% (23.0 min)	0.38% (23.0 min)

Degradant ≥0.10% is described (hyphen indicates <0.10%). Measurement was performed at $n=1$.

SS layer of the bilayer tablets markedly occurred under the humidity stress condition (40°C/75%RH in open glass vials) rather than the heat stress condition (50°C in sealed aluminum bag). The degradant found in the SS layer with a retention time of 23.0 min is an impurity, which has been characterized in terms of its chemical structure and safety. It is noteworthy that the peak areas of five of the peaks observed in the bilayer tablets increased considerably (Peaks #1–5), and that the retention times of these peaks were identical to those of the peaks found in the MB layer of the bilayer tablets (Fig. 2). This result therefore suggests that the degradants observed in the both analyses for the MB and SS layers were considered to be formed at the interface of the bilayer tablets.

Compatibility Study Simulating the Interface of Bilayer Tablets We conducted two series of compatibility studies to determine the root cause for the degradation observed in the bilayer tablets with Formulation *X*.

Part 1 study was carried out to assess the impact of the two APIs on the degradation, using the following four compact samples: MB (−)/SS (−), MB (−)/SS (+), MB (+)/SS (−) and MB (+)/SS (+). Figure 3 shows the peak areas at 210 nm of Peaks #1–5 from the four compact samples both before and after the stress storage. The peak areas of the degradants remained largely unchanged for the samples containing only MB or SS but increased in the samples containing both MB and SS. The result indicated that MB and SS were both involved in the degradation reaction. There was a slight increase of Peak #3 even in the presence of MB alone, suggesting po-

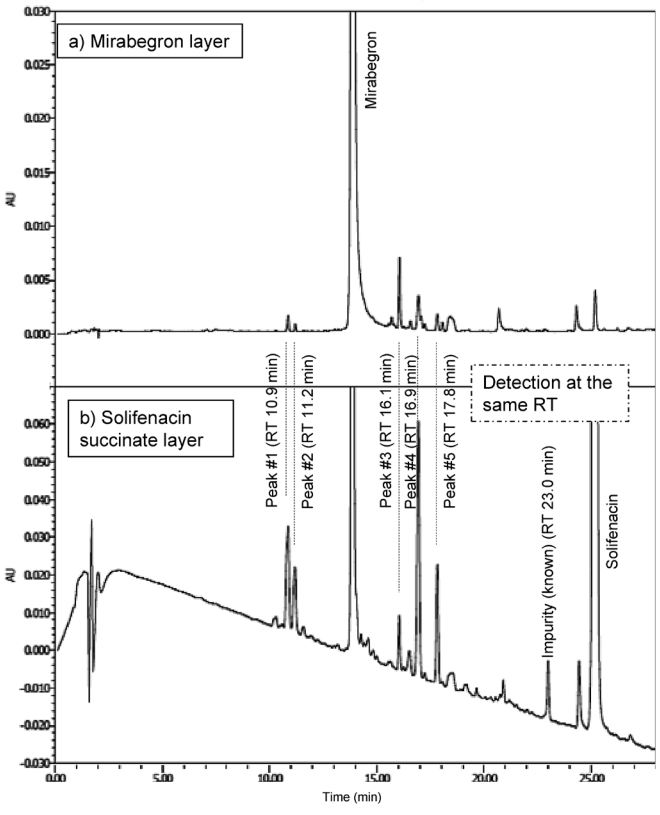

Fig. 2. Chromatograms of Degradants of (a) Mirabegron Layer and (b) Solifenacin Succinate Layer of Mirabegron/Solifenacin Succinate Bilayer Tablets with Formulation *X* (40°C/75% RH in Open Glass Vials for 1 Month)

RT means retention time.

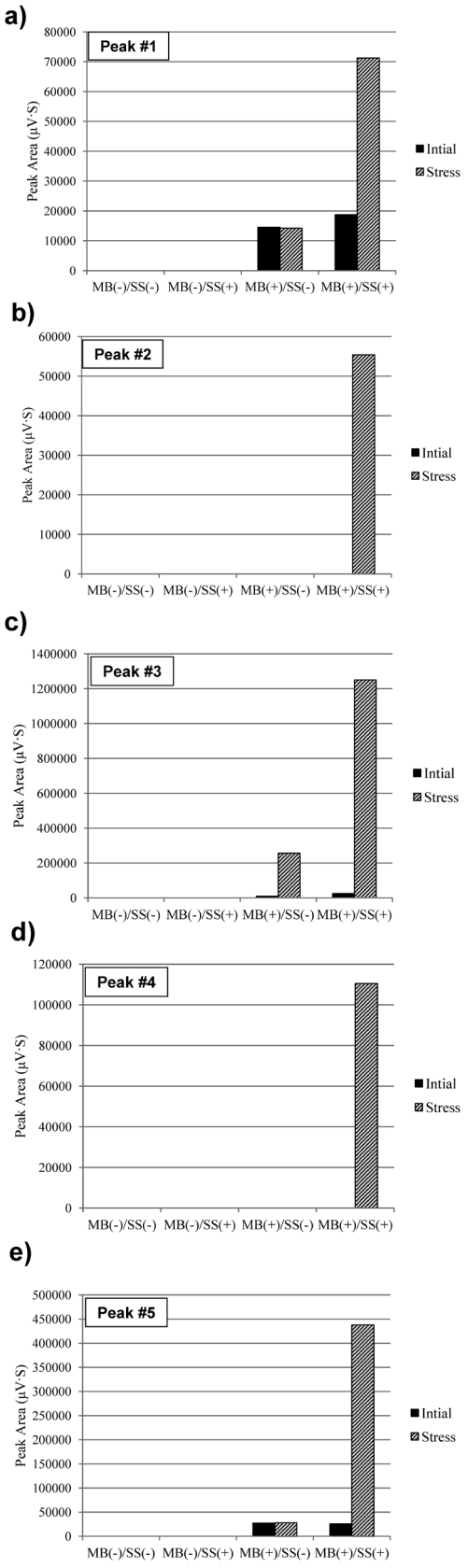

Fig. 3. Results of Compatibility Study Part 1: Peak Areas of (a) Peak #1, (b) Peak #2, (c) Peak #3, (d) Peak #4 and (e) Peak #5 before and after the Storage of Compact Samples under the Stress Condition of 40°C/75% RH for 4 d

If no peak is detected (>the peak 9000 μV∗S corresponding to 0.05% level), the peak area is depicted as zero. Measurement was performed at $n=1$.

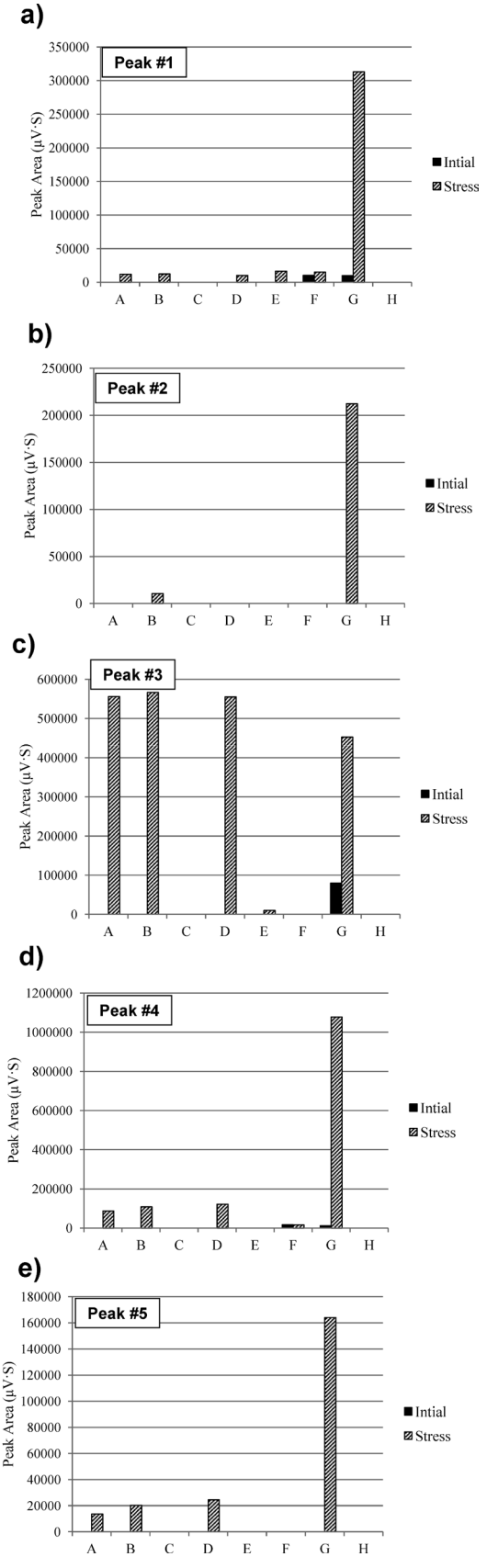

Fig. 4. Results of Compatibility Study Part 2: Peak Areas of (a) Peak #1, (b) Peak #2, (c) Peak #3, (d) Peak #4 and (e) Peak #5 before and after the Storage of Compact Samples under the Stress Condition of 60°C/75% RH for 24 h

If no peak is detected (>the peak 9000 μV∗S corresponding to 0.05% level), the peak area is depicted as zero. Measurement was performed at n=1.

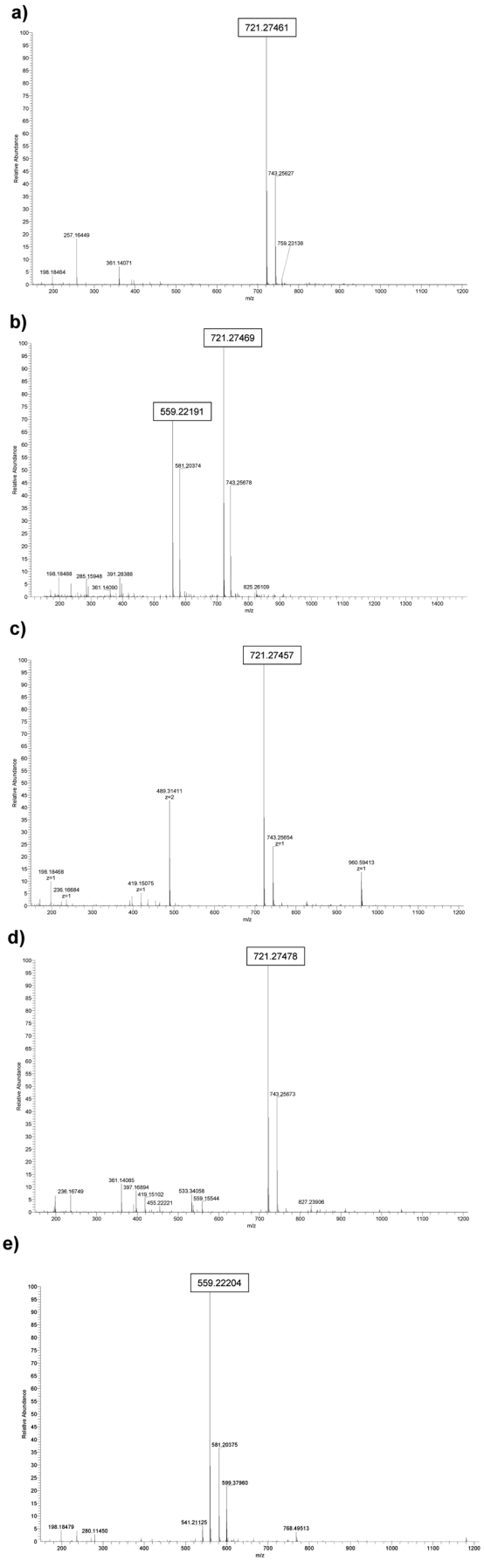

Fig. 5. MS Spectra for (a) Peak #1, (b) Peak #2, (c) Peak #3, (d) Peak #4 and (e) Peak #5

tential degradation of MB without interaction with SS.

Part 2 study was conducted to identify components related to the degradation process. The peak areas of Peaks #1–5 observed in the compact samples (components shown in Table 3) are summarized in Fig. 4. The peak areas of Peaks #1–5 increased under the stress storage condition in compact samples A, B, D and G, but did not change or were completely undetected for all of the other samples. The common components found in the samples A, B, D and G were MB, SS and maltose, which was used as a binding agent for the SS layer. None of the other excipients from the SS layer were found to be critical to the formation of the degradants. It therefore became evident that these three components were related to the degradation. A comparison of Peaks #1–5 among the eight different samples revealed that a larger increase of peak area was seen in the sample G than the samples A, B and D, except for Peak #3. In the meantime, when focusing on Peak #3, there was a comparable or less increase in the sample G compared with the samples A, B, and D. Although the root cause for the difference of this trend remains unclear, the reaction occurring among MB, SS and maltose was probably most active in the sample G due to the absence of other unrelated excipients in the matrix and second degradation of Peak #3 might have occurred during the storage, resulting in the observed decrease in the peak area of Peak #3 in sample G.

Determination of the Chemical Structures of the Degradants To develop a deeper understanding of the degradation process, we conducted high resolution HPLC-MS measurements and evaluated the resulting MS and MS/MS spectra to determine the molecular weights of the parent mass ions and the corresponding fragment ions. The MS spectra were recorded using electrospray ionization in the positive ionization mode, which gave superior results compared with the negative ionization mode.

The MS data for Peaks #1–5 are described in Fig. 5. The major MS peak observed in the spectra of Peaks #1, 2, 3 and 4 gave an *m*/*z* value of 721, whereas the major MS peak observed for Peak #5 gave an *m*/*z* value of 559. Peak #2 also contained a mass ion with an *m*/*z* value of 559, which could be attributed to the presence of another degradant at the same retention time or the fragmentation of the parent mass ion (*m*/*z* 721).

MS/MS spectra were extracted and evaluated for the major MS peaks (Fig. 6). The MS/MS fragmentation of the mass ion observed with an *m*/*z* value of 721 in Peaks #1–4 yielded two fragment ions with *m*/*z* values of 397 and 379. The MS/MS fragmentation of the mass ion observed with an *m*/*z* value of 559 in Peaks #2 and 5 also gave two fragment ions with *m*/*z* values of 397 and 379. These two fragments correspond to the molecular weights of MB ($C_{21}H_{25}O_2N_4S$) and MB minus a single molecule of water ($C_{21}H_{23}ON_4S$). These peaks can therefore be classified as MB degradants due to the presence of the basic chemical structure of MB. In contrast, there was no MS/MS peak originating from SS structure. The fragment ion with an *m*/*z* value of 559 was also found in the MS/MS spectra extracted from the mass ion with an *m*/*z* value of 721. The differences observed in the molecular weight of MB (*m*/*z* values of 397) and the peaks with to *m*/*z* values of 581 and 721 were consistent with the addition of $C_6H_{10}O_5$ (*i.e.*, a single sugar unit) or $C_{12}H_{20}O_{10}$ (*i.e.*, a double sugar unit).

Additional MS/MS spectra were evaluated to determine where the sugar units were attached to MB. In all five peaks, the MS/MS data revealed a fragmentation ion with an *m*/*z* value of 141, which is indicative of $C_5H_5ON_2S$ (*i.e.*, the aminothiazole end of MB). Peaks #1 and 2 revealed fragment ions with *m*/*z* values of 155 and 183, which were consistent with the addition of CH_2 and C_2H_2O to $C_5H_5ON_2S$, respectively.

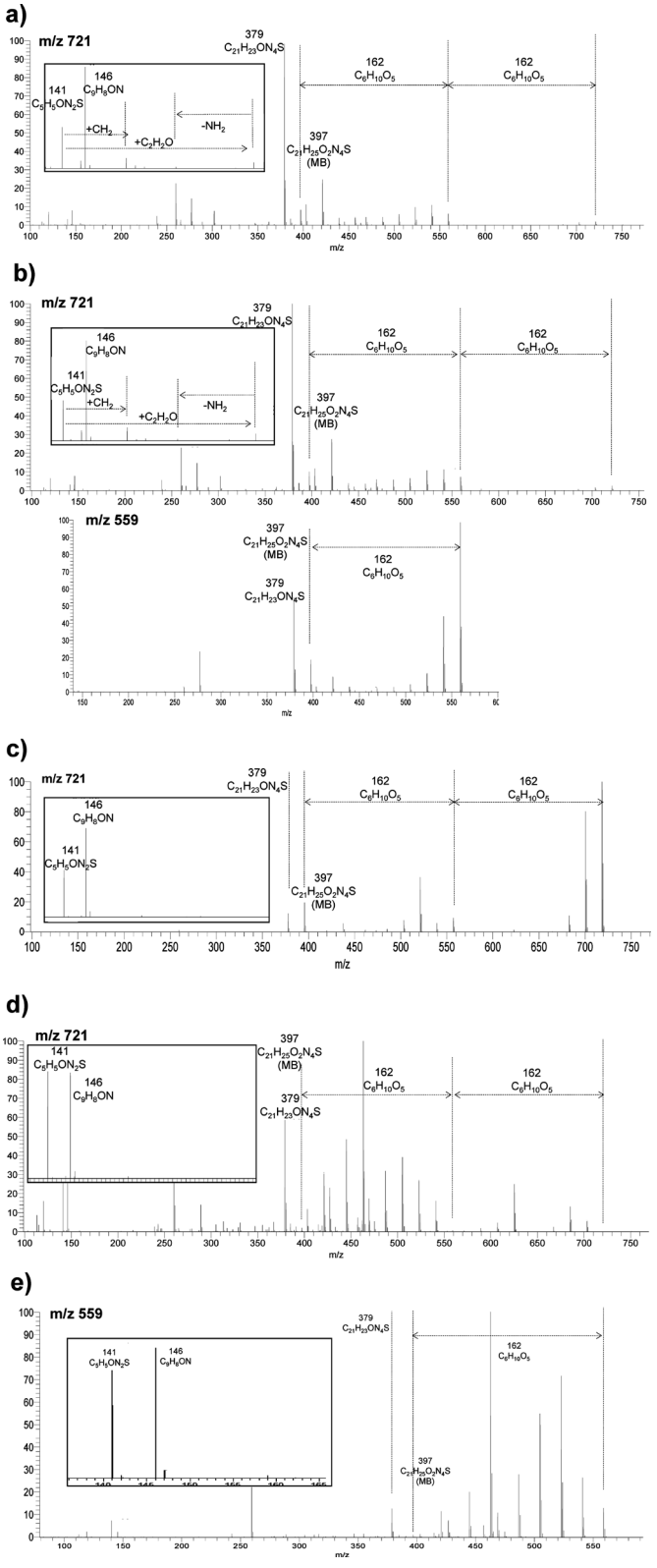

Fig. 6. MS/MS Spectra from the Main MS Peaks of (a) Peak #1, (b) Peak #2, (c) Peak #3, (d) Peak #4, and (e) Peak #5

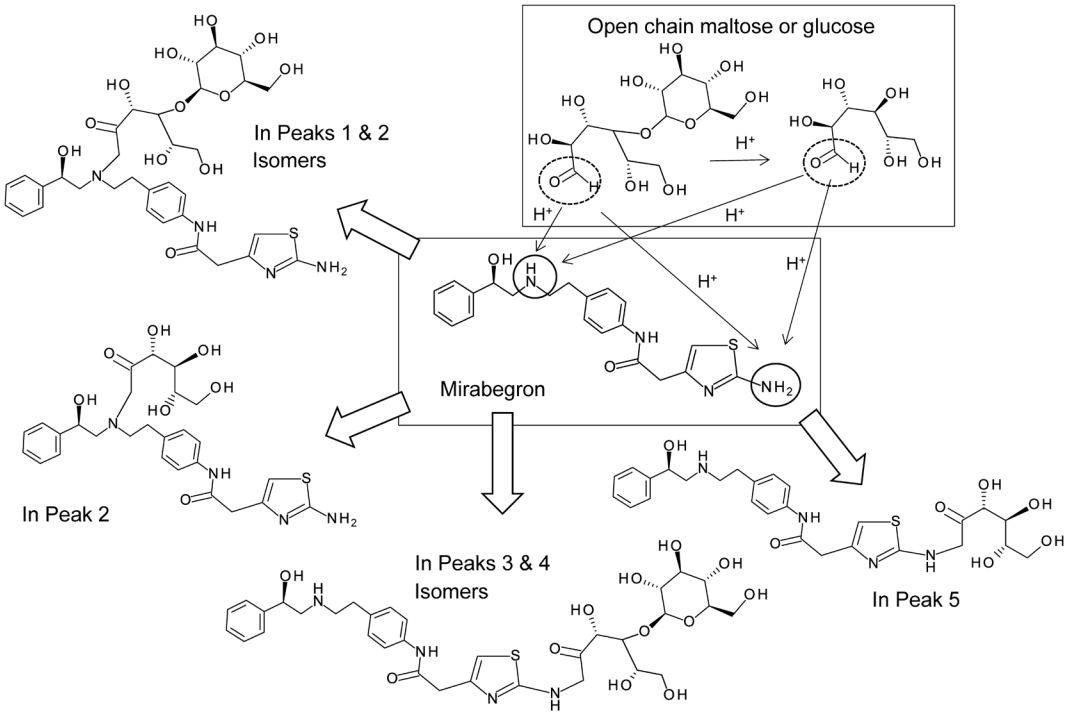

Fig. 7. Estimated Chemical Structures of Degradants and Degradation Mechanisms

Table 6. Chemical Stability Data of Mirabegron/Solifenacin Succinate Bilayer Tablets with Formulation *Y*

		Stability condition					
			Aluminum/aluminum blister			Sealed aluminum bag	Open glass vial
		Initial	25°C/60% RH (long-term)	40°C/75% RH (accelerated)		50°C (stress)	40°C/75% RH (stress)
			6 months	3 months	6 months	1 month	1 month
% Degradant (retention time)	MB layer	0.12% (18.4 min)	0.11% (18.4 min)	0.13% (18.4 min)	0.14% (18.4 min)	0.16% (18.4 min)	0.15% (18.4 min)
		—	—	—	—	—	0.20% (25.2 min)
	SS layer	—	0.13% (20.9 min)	0.14% (20.9 min)	0.14% (20.9 min)	0.10% (20.9 min)	0.13% (20.9 min)
		0.38% (23.0 min)	0.45% (23.0 min)	0.48% (23.0 min)	0.52% (23.0 min)	0.45% (23.0 min)	0.44% (23.0 min)

Degradant ≥0.10% is described (hyphen indicates <0.10%). Measurement was performed at $n=1$.

A fragment ion consistent with the desorption of NH_3 from the C_2H_2O additive was also detected at *m/z* 166. This pattern suggested that the addition of the sugar unit was occurring at a position away from the amino thiazole moiety of MB. Peaks #3, 4 and 5 also showed the fragment ion with an *m/z* value of 141, but there was no obvious pattern which could supports the presence of the primary amine, suggesting that the addition of sugar occurred at the primary amine and then the fragment ions relevant to desorption of NH_3 were not detected.

Taken together, these data suggested that the chemical structures of the degradants were consistent with the structures shown in Fig. 7.

Estimation of Degradation Mechanism The results of the compatibility study revealed that the chemical degradation only occurred in the presence of MB, SS and maltose. HPLC-MS/MS analysis demonstrated that the degradants were derived from MB bearing one or two sugar moieties. Because the HPLC-MS/MS data did not provide any information pertaining to the structure of SS, it seemed clear that SS was simply activating the degradation of MB without being degrading or reacting itself. Based on these observations, we proposed a plausible degradation mechanism (Fig. 7). SS would generate acidic conditions through its interactions with the free water molecules adsorbed from external moisture. Maltose (disaccharide) would be partially hydrolyzed under these conditions to form glucose. The acyclic forms of maltose and glucose contain a reducing end (aldehyde functionality), which would react with the primary or secondary amine of MB.[13,14] This reaction is an Amadori rearrangement, which is catalyzed by acid.[15]

Improved Formulation of MB/SS Bilayer Tablets These observations clearly demonstrated that maltose was unfavorable as an excipient for the formulation of chemically stable MB/SS bilayer tablets. As long as maltose continues to be used, the bilayer tablets may still have a risk of chemical stability through future commercial production. Given that maltose is a sugar-based binding agent, it would also most likely lead to the same degradation products *via* the proposed deg-

radation mechanism. With this in mind, we replaced maltose with the polymer-type binding agent hydroxypropyl cellulose leading to more stable formulation (Table 1).

The results for the chemical stability of Formulation *Y* are shown in Table 6. As expected, none of the degradants being formed at the interface of the bilayer tablets (Formulation *X*) were detected in the case of Formulation *Y*. No new degradants specific to Formulation *Y* were also found in the MB and SS layers. Based on the result of the accelerated and stress stability studies, the new formulation is likely to assure long-term stability for years.

A series of the experiments aimed at elucidating the mechanism responsible for the degradation of the bilayer tablet led the development of an alternative formulation strategy by the replacement of the sugar-based binding agent maltose with a polymer-type binding agent. This new strategy only required a minor change to the existing process and avoided the need to use a new technique (*e.g.*, multi-layer tablets[16]), which would have required a complicated formulation process.

Conclusion

We have provided an example of a chemical degradation problem that we recently encountered during the development of MB/SS bilayer tablets. Bilayer tableting technology is currently considered to be a useful option for the formulation of stable FDC tablets because it decreases the contact surface areas between incompatible APIs.[1,5] However, in this particular case, we still observed degradation, despite limiting the extent of the physical contact between the two APIs. We observed a considerable increase in the amount of degradants observed in our initial formulation of MB/SS bilayer tablets compared with the MB mono tablets. The results of a series of compatibility studies and mass spectroscopy analyses revealed that the degradation was caused by three factors (*i.e.*, MB, SS and maltose) and that the degradants were derivatives of MB bearing one or two sugar moieties. The mechanism responsible for the degradation in the bilayer tablets was therefore established, leading to the development of an alternative strategy for the robust formulation of MB/SS FDC tablets. With these adequate justifications, this new formulating strategy resulted in a low risk of unwanted increase of degradant during storage.

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Conflict of Interest The authors declare no conflict of interest.

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