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Stability Behaviour of Molsidomine-containing Pellet Formulations

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Abstract

Molsidomine in mixtures with different inactive ingredients has been subjected to a stability test.

The fingerprint chromatogram obtained by HPLC with diode-array detection of mixtures of molsidomine with povidone 25 revealed decomposition products; the detection wavelength of 210 nm resulted in easy detection of the degradation products. Molsidomine-containing pellets were manufactured according to a compact procedure and by applying the active ingredient to placebo pellets. Compared with the nonpareil pellet formulations, compact pellets have a considerably higher water content and undergo decomposition of the active ingredient after storage for 50 months under different conditions.

It is assumed that the decomposition of molsidomine is accelerated by the peroxide found in povidone.

The stability of molsidomine (*N*-ethoxycarbonyl-3-morpholinosydnomine) in solution has been described (Asahi et al 1971). Hydrolysis, among other conditions, results in decomposition of molsidomine and its salts by a pseudo first-order reaction. The hydrolysis of molsidomine at pH values between 4·5 and 7·5 proceeds at a constant rate. Molsidomine is unstable when exposed to light (Thoma & Kerker 1992a, b); nitrosomorpholine is the main degradation product. Measurable stabilization can be achieved by adding stabilizing agents, e.g. colorants, vanillin, toxerutin (Voegele & Laudenbach 1985, Voegele et al 1986) or PEG (Schraven et al 1994). Instability to light is not observed in solutions protected from UV radiation (Vandenbossche et al 1993).

This paper describes interactions between inactive ingredients and molsidomine. The evaluation is based on pre-formulation and long-term stability tests for pellets manufactured by granulation in a high-speed mixer (formulation 1) and by applying the active ingredient to sugar globules (formulation 2).

Materials and Methods

The particle size of the molsidomine used (Shiratori, Narashino City, Japan) was determined by image analysis (Omnikon Alpha, Pabisch, Munich, Germany) as $d = 11{\cdot}3$ μm with $n = 1{\cdot}65$. The fillers used for the pellet formulation were: sucrose (Süddeutsche Zucker, Rain am Lech, Germany), lactose (Granulac 70, Meggle, Wasserburg, Germany), microcrystalline cellulose (Avicel PH 105, FMC, Philadelphia, USA) and maize starch (extra white maize starch, Roquette, Frankfurt, Germany).

The binding agents used were povidone 25 (BASF, Ludwigshafen, Germany) and hydroxypropyl(methylcellulose) (Pharmacoat 603, Shin-Etsu, Tokyo, Japan).

Flowability was improved by use of high-dispersion silica (Aerosil 200, Penta, Aschaffenburg, Germany). Sugar globules with a diameter of 0·71–0·85 mm were composed of 75% sucrose and 25% maize starch (Werner, Tornesch, Germany).

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Manufacturing of the powdered mixture for pre-formulation tests

The components for the pre-formulation tests (Table 1) were mixed for 30 min in a cube mixer (Erweka AR 400, Heusenstamm, Germany).

Pellet manufacture

The formulation-1 pellets (Table 2) were manufactured in a Henschel FM 10 high-speed mixer (Henschel-Rheinstahl, Kassel, Germany). The powder components were pelletized in the mixer at 400 rev min^{-1} with 250 mL isopropanol at a spray rate of approximately 100 mL min^{-1} in approximately 3 min. Drying ensued for 20 min at 60°C in a fluid bed (Aeromatic Strea-1, Niro-Aeromatic, Bubendorf, Switzerland). Manual sieving was used to separate the particle fraction between 0·71 mm and 1·25 mm.

For the formulation-2 pellets, the sugar globules were put in the Henschel mixer and moistened at 400 rev min^{-1} with isopropanol or with a 60 : 40 (v/v) mixture of isopropanol and demineralized water. This was then sprinkled with a mixture of the remaining components (Erweka AR 400). The moistening and sprinkling process was repeated 20 times using a total of 80 g isopropanol or 60 g of the 60 : 40 isopropanol-water mixture, the overall process time being 20 min. Subsequently, the pellets were dried for 20 min in the Aeromatic Strea-1, encapsulated in size 1 capsules and blister-packed in aluminium-PVC (DPN 740, Noack, Karlsruhe, Germany).

Storage for stability testing

The blister packs without conditioned moisture conditions (50°C) were stored in a TV 40 UL drying oven (Memmert, Schwabach, Germany); those with moisture conditioning (25°C and 60% relative humidity or 30°C and 70% relative humidity) in an HC 0033 universal climatic chamber (Heraeus, Balingen, Germany). The powdered mixtures were stored for 4 weeks in open Petri dishes at 30°C and 70% relative humidity or at 50°C in screw-cap vials.

Table 1. Powdered mixtures for pre-formulation tests.

	Number				
	1	2	3	4	5
Molsidomine (%)	10·0	22·5	45·0	10·0	10·0
Filler (%)					
Avicel PH 105	38·0	–	–	–	–
Maize starch	40·0	77·5	–	–	–
Binding agent (%)					
Povidone 25	12·0	–	55·0	–	1
Pharmacoat 603	–	–	–	1	–
Other (%)					
Placebo/ powdered pellets	–	–	–	89·0	89·0

Analysis

For sample preparation, active ingredient or pellets (approximately 100 mg) were accurately weighed into a 100 mL volumetric flask; mobile phase was added and the mixture shaken until the pellets disintegrated; the flask was then filled to volume with mobile phase. After centrifugation for 10 min at 4000 rev min^{-1}, the solution was diluted with mobile phase so that the sample used for determination of content contained approximately 280 ng/20 μL and the sample for the purity assay approximately 5000 ng molsidomine/20 μL.

To determine a sensitive and selective measuring wavelength for the degradation product, isograms were prepared in the measuring range 190–370 nm. The HPLC equipment consisted of a solvent pump (LKB 2150, Bromma, Sweden), a diode-array detector (LKB 2140 Rapid) and a variable-wavelength detector (Spectra-Physics 100, Darmstadt, Germany), a reversed-phase column (Nucleosil 100 C_{18}, Macherey-Nagel, Düren, Germany), and an automatic integrating system (LKB WSEG software). A 30:70 (v/v) mixture of methanol and phosphate buffer (0·01 M in distilled water) was used as mobile phase. Because of the greater measurement sensitivity of a grid monochromator detector, after determining the appropriate wavelength of 210 nm for the degradation product, the samples were analysed by means of an SP UV 1000 UV detector (Spectra-Physics), an SP 2000 solvent pump (Spectra-Physics) and a ChromJet analysis unit (Spectra-Physics). Consequently, the determination of content was also performed on non-decomposed substances at a wavelength of 315 nm. The mobile phase was the same as before, but in the ratio 50:50 (v/v). Both methods of determination obtained a linear relationship between response and molsidomine concentrations from 6 to 800 ng/20 μL at a system precision of better than 0·25% (n = 16) for the injection concentration 100 ng/20 μL. The determination of water content was performed by the Karl Fischer method (KF-Titrino 701/703, Metrohm, Herizau, Switzerland).

Results and Discussion

Preformulation tests

During storage in open Petri dishes for 4 weeks at 30°C and 70% relative humidity or in screw-cap vials at 50°C, no changes were observed in molsidomine in the measuring range 190–370 nm. Visual inspection of mixtures 1 and 3 revealed yellowish discoloration and high conglutination after storage at 30°C and 70% relative humidity.

In the fingerprint chromatogram, molsidomine fission products were easily detected at 210 nm. Under both sets of storage conditions, degradation products at retention times of 2–3 min were discernible to a large extent in mixtures 1 and 3 and to a small extent in mixture 2, compared with pure molsidomine. This was evident after 4 weeks, storage (30°C and 70% relative humidity), both in the fingerprint of the diode array chromatogram (Fig. 1) and in the chromatograms recorded by the grid monochromator. Fig. 2 shows the chromatogram of the laboratory standard, molsidomine and the eluent, stored for 4 weeks at 50°C, and Fig. 3 the chromatogram for mixtures 1–3, stored for 4 weeks at 50°C in screw-cap vials; decomposition products are apparent at retention times of 3·5–4·5 min.

After storage for 4 weeks at 50°C in screw-cap vials, mixture 1 had a water content of 8·9%, mixture 2 of 8·5%, mixture 3 of 5·2%, mixture 4 of 1·9% and mixture 5 of 2·1%.

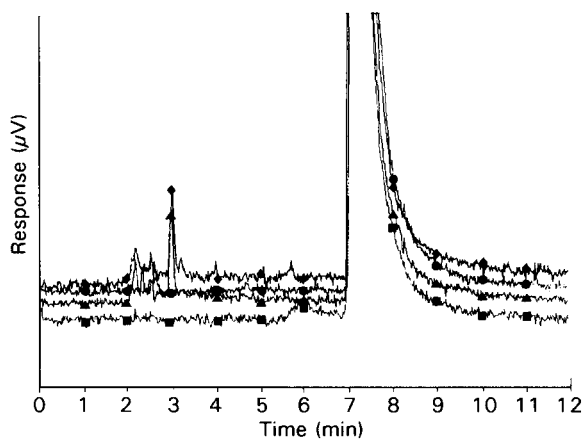

FIG. 1. Fingerprint chromatogram of mixture 2 and molsidomine; detection, 210 nm. ◆, 50°C (mixture 2); ▲, 30°C and 70% relative humidity (mixture 2); ●, 50°C (molsidomine); ■, 30°C and 70% relative humidity (molsidomine); storage time, 4 weeks.

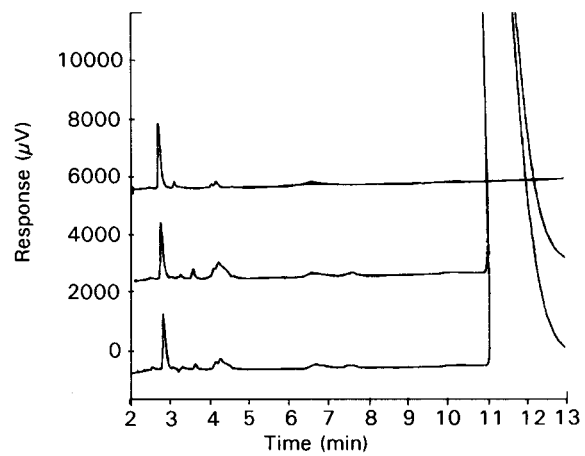

FIG. 2. Chromatogram of the eluent, laboratory standard and molsidomine; detection, 210 nm. Storage time, 4 weeks; temperature, 50°C. Upper, eluent; middle, molsidomine used for pre-formulation/formulation; lower, laboratory standard.

Table 2. Molsidomine pellet formulations.

	Formulation					
	1 Pellets, manufactured in the high-speed mixer		2 Pellets based on sugar globules		3 Pellets based on sugar globules	
	(%)	(g/batch)	(%)	(g/batch)	(%)	(g/batch)
Molsidomine	10·0	100·0	10·00	150·0	10·0	150·0
Maize starch	40·0	400·0	–	–	–	–
Avicel PH 105	38·0	380·0	–	–	–	–
Povidone 25	12·0	120·0	1·13	16·9	–	–
Pharmacoat 603	–	–			0·53	8·0
Aerosil 200	–	–	0·11	1·6	0·11	1·6
Placebo pellets	–	–	88·77	1331·5	89·36	1340·4
Total		1000·0		1500·0		1500·0

Table 3. Initial and remaining molsidomine content in pellets manufactured in the high-speed mixer and stored for 50 months under different conditions (formulation 1).

Storage conditions		Molsidomine content (mg/80·0 mg)			Actual value/ required value (%)			Water content (%)		
Temperature (°C)	Relative humidity (%)	Initial	Remaining	Mean		Mean	s.d.		Mean	s.d.
50	–		8·04		92·2			6·3		
			7·95	8·04	91·2	92·6	0·8	7·2	7·2	12·5
			8·07		92·6			8·1		
25	60		7·35		84·3			9·2		
			7·46	7·42	85·6	85·1	0·8	9·1	9·1	0·6
			7·44		85·3			9·1		
30	70		6·53		74·9			8·3		
			6·73	6·58	77·2	75·5	2·0	8·3	8·3	0·7
			6·48		74·3			8·2		

Long-term stability tests

Molsidomine proved to be unstable in pellets manufactured by a compact procedure, as for formulation 1, and stored over a period of 50 months, this instability being at its lowest when the pellets were stored dry at 50°C (Table 3). Molsidomine decomposition was greater at 25°C or 30°C under the influence of moisture. After storage for 50 months, the water content was between 7·2 and 9·1% under all conditions. The active ingredient did not decompose in pellets in which the active ingredient was applied to placebo globules, irrespective of whether povidone or hydroxypropyl (methylcellulose) was used as the binding agent (Table 4). The water content of the samples stored for longer than 50 months was always below 2%.

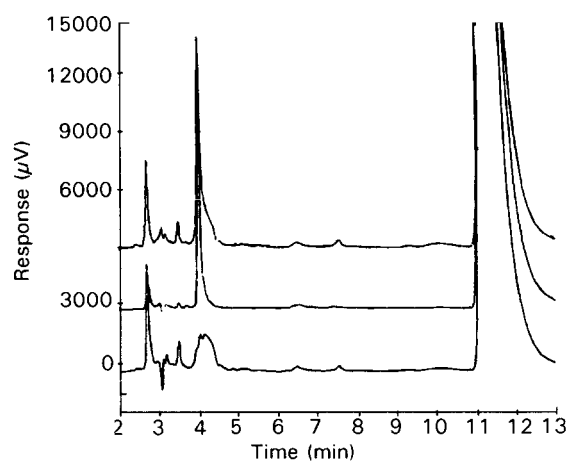

FIG. 3. Chromatogram of mixtures 1–3; detection, 210 nm. Storage time, 4 weeks; temperature, 50°C. Upper, formulation 1; middle, formulation 2; lower, formulation 3.

Discussion

In the pre-formulation test, mixtures with povidone 25 absorbed more water and resulted in more evidence of instability in the fingerprint chromatogram. The chromatogram obtained from the binary mixture of molsidomine and maize starch revealed slight indications of instability despite high water absorption. Mixtures prepared according to a nonpareil pellet formulation with a small amount of povidone 25 absorbed little water and showed no signs of molsidomine instability with the inactive ingredients used.

Molsidomine shows instability in pellet formulations that

Table 4. Initial content/remaining content of molsidomine in pellets based on sugar globules, stored for 50 months under different conditions.

Formulation	Storage conditions		Molsidomine content (mg/80·0 mg)			Actual value/required value (%)			Water content (%)		
	Temperature	Relative humidity	Initial	Remaining	Mean		Mean	s.d.		Mean	s.d.
	(°C)	(%)									
2	50	–	7·94	8·04		101·3			0·8		
				7·85	7·94	98·9	100·1	1·2	1·0	1·0	25·2
				7·94		100·0			1·3		
	25	60		8·09		101·9			1·6		
				7·82	8·06	98·5	101·6	2·9	1·8	1·7	6·8
				7·94		104·3			1·8		
	30	70		8·11		102·1			1·5		
				7·88	7·96	99·2	100·2	1·6	1·8	1·7	9·0
				7·89		99·4			1·7		
3	50	–	7·87	8·01		101·8			0·8		
				8·02	8·04	101·9	102·2	0·5	1·1	1·1	27·0
				8·09		102·8			1·4		
	25	–		7·98		101·4			1·3		
				8·40	8·08	106·7	102·6	3·5	1·6	1·5	10·2
				7·85		99·8			1·5		
	30	70		7·66		97·3			1·3		
				7·92	7·82	100·6	99·4	1·8	1·5	1·5	10·2
				7·89		100·3			1·6		

contain povidone and have a high water absorption capacity. If the active ingredient is applied to placebo pellets composed of sucrose (75%) and maize starch (25%), there is no instability, even when povidone is used as the binding agent. Pellets formulated in this way contain a low percentage of povidone and only absorb small amounts of water, even when stored at 30°C and 70% relative humidity.

From the results of the pre-formulation tests, it is assumed that the peroxide contained in povidone 25 accelerates the decomposition. In conformity with harmonized pharmacopoeial requirements, the peroxide content in povidone 25 is limited to 400 ppm, expressed as hydrogen peroxide.

Further studies should be performed to determine whether a combination of moisture and high temperatures increases the peroxide content in povidone 25 during storage; this would explain the greater decomposition of molsidomine at higher moisture levels and storage temperatures, but at comparable bulk moisture of the stored pellets.

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