EXHIBIT “R”

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Report

Drug-Excipient Incompatibility Studies of the Dipeptide Angiotensin-Converting Enzyme Inhibitor, Moexipril Hydrochloride: Dry Powder vs Wet Granulation

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The drug-excipient incompatibility screen for moexipril hydrochloride (1) using various isothermal stress methods is reported herein. It was found that most of the commonly used fillers, disintegrants, lubricants, glidants, and coating agents were incompatible with 1 in dry powder mixtures; moisture and basic (or alkalizing) agents were determined to be the dominant destabilizing factors. In wet granulations, basic agents, however, were found to suppress drug degradation even in the presence of moisture. Supported by the product distribution studies, the stabilization is proposed to involve the neutralization of the acidic drug by the basic excipients.

KEY WORDS: incompatibility screen; moexipril; stability; solid state.

INTRODUCTION

Drug-excipient incompatibility testing is an important process to the development of a stable solid dosage form (1,2). Various accelerated protocols have been proposed for incompatibility testing throughout the years; short-comings are, however, often unavoidable because these protocols have to accommodate the time constraint and limited drug quantity, especially at the early stage of the developmental program. For example, differential scanning calorimetry (DSC), which measures energy transfer as a function of temperature, has been applied in excipient incompatibility screenings for cephalexin (3), ampicillin (4), and clenbuterol (5). Only milligram quantities of drug are typically needed for DSC measurements. The interpretation of DSC thermograms of drug-excipient mixtures, however, can sometimes be difficult and the conclusions based on DSC results alone are often misleading (6,7). This is due primarily to the high-temperature conditions required and the lack of moisture stress in conducting DSC experiments.

The isothermal stress of the simple drug-excipient mixtures stored in sealed containers with or without added moisture is probably the most commonly used incompatibility testing method. The isothermal stress method is more time-consuming and requires quantitative analysis of the drug and/or its degradation products. Although more applicable, the isothermal stress still does not address properly the effect of wet granulation, often used in solid formulations.

Moexipril hydrochloride belongs to a class of *N*-carboxyalkyl dipeptide angiotensin converting enzyme (ACE) inhibitors (8). Like many of these dipeptide ACE inhibitors, moexipril hydrochloride is unstable in the solid state, especially in the presence of excipients. In this study, the incompatibility of both dry powder and wet granulations of moexipril hydrochloride-excipient(s) mixtures are evaluated by HPLC using the isothermal stress protocol.

EXPERIMENTAL

Chemicals

Moexipril hydrochloride (1), moexipril diketopiperazine (DKP; 2), and moexipril diacid (3) were obtained from the Institute of Organic Chemistry, Syntex Research. The excipients used were either analytical grade or USP grade and were triturated if necessary. HPLC-grade acetonitrile and tetrahydrofuran along with nanopure water were used to prepare the mobile phase.

HPLC Method

The reverse-phase HPLC method employed an Altex Ultrasphere-ODS 50-μ, 4.6-mm-i.d. × 250-mm column and a mobile phase of ammonium phosphate monobasic buffer (0.05 *M*, pH 2.0)/acetonitrile/tetrahydrofuran (55/35/10). The flow rate was 1.0 ml/min with UV detection at 220 nm. Typical injections contained ~2–3 μg of the drug with the injection volume of 50 μl.

Dry Powder Mixtures

Approximately 125 mg of both the moexipril hydrochlo-

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ride and a selected excipient was accurately weighed into a 10-ml clear volumetric flask, followed by mixing on a Vortex mixer for ~4 min. Flasks containing various drug-excipient mixtures were then placed in a plastic ointment container with humidity controlled by a saturated solution of sodium bromide (50% RH), potassium acetate (21% RH), or lithium chloride (11% RH). The containers were tightly fitted with a screw-cap lid, sealed with tape, and then placed in a 60°C oven for 13 days.

Sample preparation consisted of adding methanol (moexipril hydrochloride and its degradation products are very soluble in methanol, whereas most excipients used are less soluble in methanol than in water) up to the 10-ml mark and sonicating for 5 min. The methanol suspension was centrifuged for 5 minutes at 2000 rpm and the clear supernatant was diluted with 1:1 water:methanol to ~30 μg/ml drug concentration. The amount of drug was quantitated by HPLC using external standards.

Drug-Excipient Mixtures in Closed Containers

Approximately 125 mg, of both the drug and a selected excipient, was accurately weighed into a 5-ml clear screw-capped vial, followed by mixing on a Vortex mixer for ~4 min. To half of the dry powder mixtures was added an aliquot (~30 μl) of doubly distilled water to achieve a final water concentration of 15% (w/w). The mixture was then mixed on a Vortex mixer for 5 min to obtain a homogeneous mixture. All vials with or without added water were then sealed with screw caps and placed in a 60°C oven for 13 days.

Sample preparation consisted of transferring the entire contents of each glass vial into a separate 100-ml clear volumetric flask, adding water up to ~80 ml, and sonicating for 5 min. The flask was then q.s. up to the mark with water and centrifuged at 2000 rpm for 5 min. The clear supernatant was diluted 25-fold with water and assayed by HPLC.

Table I. Results of Moexipril Hydrochloride:Excipient (1:1) Dry Powder Incompatibility Studies at 60°C for 13 Days

		% moexipril remaining		
Excipient	Function	50% RH	21% RH	11% RH
Moexipril, only		96.3	98.1	98.1
Lactose	Filler	93.2	98.6	98.3
Microcrystalline cellulose	Filler/Binder	92.9	98.6	98.1
Pregelatinized starch	Filler	89.8		
Crospovidone	Distintegrant	85.6		
Primojel	Disintegrant	<40		
AC-Di-Sol	Disintegrant	<40		
Stearic acid	Lubricant	92.7		
Sterotex	Lubricant	97.9		
Mg stearate	Lubricant	<10		
Eudragit L100-55	Coating	93.4	99.7	98.3
Ethyl cellulose	Coating	88.7		
Syloid 63	Glidant	85.1		
TALC	Glidant	81.0		

Drug-Lactose-Excipient (5/45/50) Dry Powder and Wet Granulations

The dry and wet granulations were prepared in a mortar by slowly adding 1.0 g of drug to 9.0 g of lactose with constant mixing using a pestle. The 10.0 g of the excipient was then slowly added into the sample with constant mixing until a homogeneous mixture was obtained. At this point 1.0 g was taken out and used as the dry granulation. To the remaining 19 g was added 1.3 ml of water (7% by weight) drop by drop with constant mixing until the mixture appeared homogeneous. This wet granulation was then dired in a 60°C vacuum oven for 6 hr. The dried wet granulated material was again mixed in the mortar using a pestle to obtain a homogeneous fine powder.

Both the dry and the wet granulations were set up on stability testing by accurately weighing ~100 mg each of the granulations into clear 10-ml volumetric flasks. The flasks were then placed in plastic ointment containers that contained either saturated sodium bromide solution (50% RH) or Drierite (anhydrous $CaSO_4$, which is denoted 0% RH for simplicity). The ointment containers were then sealed with a screw-cap lid and placed in a 60°C oven for 13 days.

Sample preparation consisted of adding 10 ml of methanol up to the mark and sonicating for 5 min. The suspension

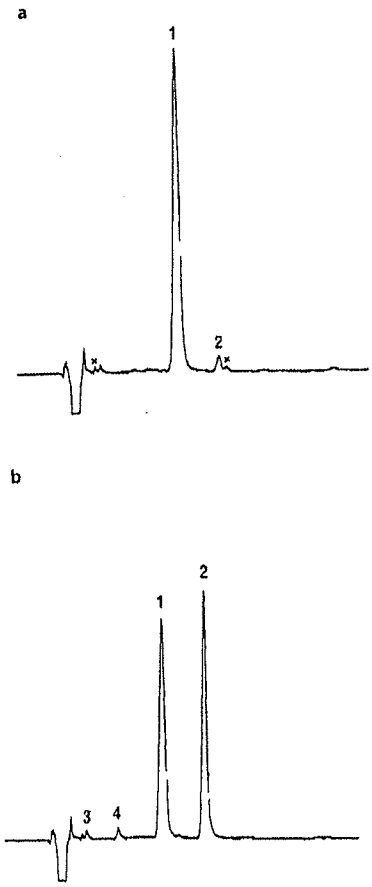


Fig. 1. HPLC chromatograms of the degraded samples of (a) moexipril hydrochloride and (b) a 1:1 mixture of moexipril hydrochloride and Primojel.

was then centrifuged at 2000 rpm for 5 min. The clear supernate was diluted 20-fold with water and assayed by HPLC.

RESULTS AND DISCUSSION

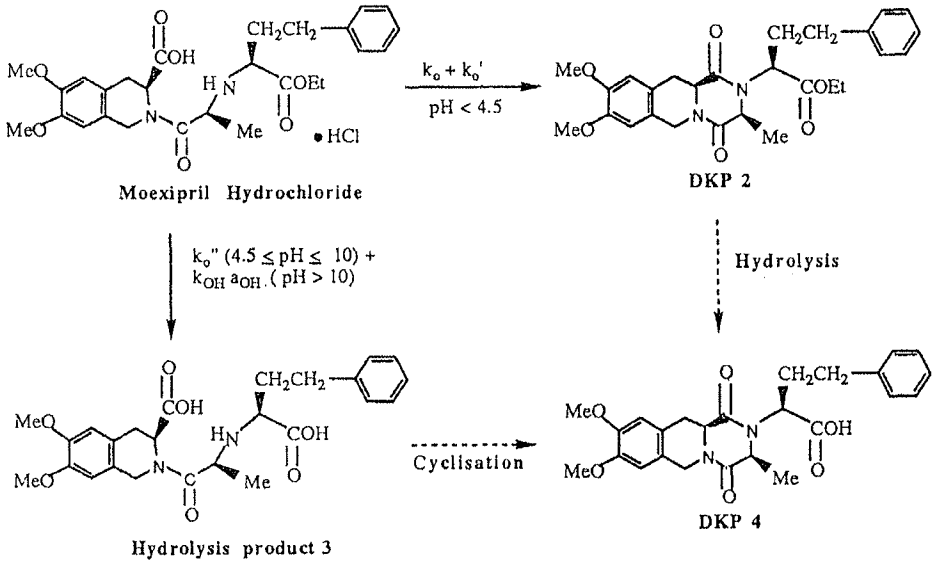
Stability of Dry Powder Mixtures at Controlled Relative Humidity (RH)

The stability of moexipril hydrochloride and its 1:1 dry powder mixtures with commonly used fillers, disintegrants, lubricants, glidants, and coating agents was first evaluated at 60°C for 13 days using a stability specific HPLC method. The results are summarized in Table I. At 50% RH, the drug by itself showed 96.3% remaining (or 3.7% degradation), and except for Sterotex, all excipients accelerated drug degradation as indicated by the lower drug recovery (85–10% remaining). The drug was significantly more stable in a lower-moisture environment. For example, the raw material showed less degradation at 21 or 11% RH (98.1% remaining or 1.9% degradation) than at 50% RH. Similarly, all 1:1 mixtures of drug:excipient studied showed at least twice as much degradation at 50% RH than at or below 21% RH (Table I).

The major degradation product of the drug raw material (Fig. 1a) and all dry powder mixes was the DKP 2. Small amounts of hydrolysis product 3 and the diacid analogue of DKP 2, 4, were also formed with highly incompatible excipients such as Primojel (Fig. 1b) and magnesium stearate.

Rationale for Use of Basic Excipients as Potential Stabilizers

Intramolecular aminolysis leading to DKP formation for *n*-carboxyalkyl dipeptide ACE inhibitors is a common stability problem encountered in formulating these agents. To avoid such degradation, low-humidity conditions have been recommended for manufacturing enalapril tablets (9,10). In solution, the DKP formation rate was extremely sensitive to pH (11–13). For example, we have shown that moexipril hydrochloride in aqueous solution (see Scheme I) degraded mainly to DKP 2 via spontaneous k_o and k_o' cyclization processes at pH values below 4.5 (11). At pH values between 4.5 and 10, moexipril hydrochloride degradation rate was about 10 times slower than that in acid and the major product formed was diacid 3 via a spontaneous ester hydrolysis k_o'' process. At pH values above 10, the degradation rate increased linearly with the activity of hydroxide ion (a_{OH}) due to the specific base-catalyzed k_{OH} ester hydrolysis process (see Scheme I).



Scheme I

Thus, even though it was well documented in the literature that acids and bases are generally incompatible in the solid state (1), the solution data suggested that basic excipients may provide a stabilizing effect to moexipril in the solid state by neutralizing, at the reaction site, the acidic nature of the hydrochloride salt.

Drug Stability in the Presence of Basic Excipients

Based on the above rationale, various basic excipients were also evaluated with the drug in dry powder form at 60°C and 50% RH. However, inspection of the results summarized in Table II revealed that all alkalyzing agents used accelerated drug degradation significantly (<57% remaining). That basicity was a dominant destabilizing factor also explained the much better compatiblity observed for stearic acid (>85% remaining) as compared to magnesium stearate (<10% remaining) (Table I). These results demonstrated that the expected neutralization of the drug by the alkalyzing agents was not accomplished in dry powder form.

To investigate further the effect of acidity/basicity of the excipients on the stablity of moexipril hydrochloride, the closed-container isothermal stress system with or without

Table II. Effect of Alkalizing Agents on the Stability of Moexipril Hydrochloride in Dry Powder Mixes After Storage at 60°C and 50% RH for 13 Days

Excipient	% moexipril remaining
Sodium bicarbonate	52.8
Sodium citrate	56.7
Calcium carbonate	53.4
Sodium benzoate	<10

added water was used (see Experimental). The results are summarized in Table III. The raw material alone showed no apparent degradation in sealed containers after storage at 60°C for 13 days. In the presence of 15% added water the drug degraded to 16% remaining. A similar trend in reactivity was also found when neutral (lactose) or acidic (ascorbic acid and citric acid) excipients were used, as 15% added water destabilized the drug-excipient mixes significantly (Table III). The major products formed from these nonbasic drug-excipient mixes as well as drug alone in the presence of water were DKP 2, hydrolysis product 3, and the diacid analogue of DKP 2, 4 (Table III). Since the neutral and acidic excipients used would not significantly affect the pH of moexipril hydrochloride in solid state, the formation of compound 3 must come from acid-catalyzed ester hydrolysis, a minor process in aqueous solution at pH below 4 (11).

Without added water, the basic excipients (sodium bicarbonate, sodium carbonate, and calcium carbonate) also accelerated the drug degradation in sealed containers (Table III). In the presence of 15% added water, these basic excipient-drug mixtures, however, showed less degradation (>50% remaining) compared to drug alone (16% remaining). Further, water was found to have no apparent effect on the stability of the drug:sodium carbonate mixture. With or without added water, the major product for these basic excipient-drug mixtures in closed containers was DKP 2. The absence of the hydrolysis product 3 therefore indicates that the pH values of basic excipent-drug mixtures were higher at the reaction site so that the acid catalyzed hydrolysis process observed for the nonbasic excipient-drug mixtures (in the presence of 15% water) was suppressed. These closed-container results therefore demonstrated the potential benefit of using basic excipients in stabilizing the drug especially in the presence of moisture, even though moisture and basicity were identified to be the two most devastating factors to drug stability in dry powder constant-humidity studies (Table I).

Effect of Wet Granulation

The above conclusion led to the third screening study of drug:excipient mixtures, where small-scale wet granulated drug/lactose/excipient (5/45/50) materials were used (see Experimental). Lactose was added to replace 45% of the drug in the granulations because of the limited drug quantity at the time of the investigation. The wet granulated drug/lactose/ascorbic acid mixture was found to be stable at 0% RH (98.7% remaining) but degraded to 76.2% remaining at 50% RH and 60°C after 13 days (Table IV). The dry powder of the same mixture (control) also showed degradation at 50% RH but to a smaller extent (91.2% remaining). Wet granulation therefore has no stabilizing effect to drug stability when an acidic excipient is used. With either wet granulation or dry powder, DKP 2 was the major product for this mixture.

As expected, the dry powder mixtures of drug/lactose/sodium bicarbonate, sodium carbonate, or calcium carbonate, were not stable at either 50 or 0% RH (Table IV).

Table III. Stability Results of Moexipril Hydrochloride:Excipient (1:1) Dry Powder in Sealed Vials with and Without Added 15% Water After Exposure to 60°C for 13 Days

			Degradation products		
Excipient	w/wo[a]	% moexipril remaining	% cyclization to DKP 2	% Hydrolysis to diacid 3	% DKP of diacid, 4
Moexipril, only	wo	99.4	<1	—	
	w	16.0	13.6	38.9	20
Lactose	wo	99.3	<1	—	
	w	30.3	30.9	23.4	
Ascorbic acid	wo	99.0	~1	—	
	w	18.0	15.9	26.7	42
Citric acid	wo	99.4	~1	—	
	w	80.8	4.0	12.7	
Sodium bicarbonate	wo	84.5	15.3	—	
	w	51.8	38.8	4.2	5
Sodium carbonate	wo	96.0	3.5	—	
	w	94.7	5.1	~1	
Calcium carbonate	wo	86.8	13.5	—	
	w	51.2	40.1	3.4	

[a] wo, without added water; w, with added 15% water.

Table IV. Stability Results of Wet Granulated and Dry Powder Mixes of (5/45/50) Moexipril/Lactose/Excipient After Exposure to 60°C and 50 or 0% RH for 13 Days

Excipient	W/D[a]	% RH	% moexipril remaining	Degradation products: % cyclization to DKP 2	Degradation products: % Hydrolysis to diacid 3
Ascorbic acid	D	50	91.2	8.6	—
	W	50	76.2	18.7	4.4
	D	0	98.0	2.1	—
	W	0	98.7	~1	—
Sodium bicarbonate	D	50	61.2	24.9	4.9
	W	50	98.6	0.5	0.4
	D	0	70.9	26.2	—
	W	0	99.0	2.2	0.8
Sodium carbonate	D	50	25.8	70.1	2.4
	W	50	97.9	<1	2.2
	D	0	78.2	21.7	
	W	0	99.1	0.4	0.4
Calcium carbonate	D	50	42.2	43.2	5.7
	W	50	91.3	4.8	3.9
	D	0	91.2	14.3	—
	W	0	91.1	7.6	1.4

[a] W, wet granulated; D, dry powder mix.

The wet granulated material in the presence of these basic excipients, however, was much more stable than the dry powder, especially at 50% RH. Further, moisture appeared to have no effect on these wet granulated mixtures, as similar stability was observed at 50 and 0% RH (Table IV). The product formed in the presence of basic excipients shifted from largely DKP 2 for dry powder mixtures to largely DKP 2 and hydrolysis product 3 for wet granulated materials. The appearance of the latter product clearly demonstrates the neutralizing effect of the basic excipients on drug raw material in wet granulations (see Scheme I).

CONCLUSIONS

Via wet granulation, alkalizing agents were found to be effective in stabilizing moexipril hydrochloride in the solid state. Supported by the product distribution profile, the stabilization is postulated to result from the neutralization of the acidic drug by basic excipients at the outer surface of the granulated material. It is also possible that a portion of the moexipril hydrochloride was converted to the cation salts via granulation and these cation salts degraded much slower in the solid state. The stabilization of moexipril hydrochloride by basic excipients, however, was not revealed in conventional drug-excipient screening studies where dry powder mixtures were used.

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