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COMMUNICATION

Development of a Lyophilized Formulation for (R,R)-Formoterol (L)-Tartrate

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ABSTRACT

(R,R)-formoterol is a β-agonist for inhalation. Aqueous instability suggested the need for a reconstitutable lyophilized dosage form. The objective of these studies was to devise a stable, rapid-dissolving, therapeutically compatible dosage form. The effects of diluents and residual moisture on the stability of thermally stressed formoterol formulations were investigated. Drug and various excipients (acetate, lactose, and mannitol) were lyophilized and placed in humidity chambers (0 to 90% relative humidity) at 25 to 50°C. Stability was characterized by time-dependent changes using HPLC, pH, and XRD. Residual moistures were determined by Karl Fisher methods. Regression models were developed to quantify the effects of formulation and environmental variation on drug stability. Solid-state instability was observed as a function of high residual moisture and diluent type. Although the residual moistures in mannitol formulations were typically below 1%, the degradation rate (50°C) varied from 2 to 10 mcg/day, which was 1.3- to 20-fold high than observed for lactose formulations under the same relative humidity conditions. At high relative humidity, the presence of acetate significantly increased the degradation rate ($p < 0.04$). The critical residual moisture content for lactose formulations was 3%. The amount of lactose was optimized by evaluating the degradation over the temperature range 25 to 50°C.

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Mannitol and acetate were shown to be unsuitable excipients, and an optimal lactose amount was 50 mg for vials containing 50 mcg of drug.
KEY WORDS: Lyophilization; Solid state instability; Formoterol, lactose formulation.

INTRODUCTION

(R,R)-formoterol is the single-isomer version of racemic β-agonist formoterol (1–3). In early clinical trials, (R,R)-formoterol exhibited dose-dependent increases in FEV_1 immediately after administration and 24-hr postdose. Patients receiving higher doses of (R,R)-formoterol showed improvements in FEV_1 greater than 15% (4). Aqueous instability suggested the utility of a reconstitutable lyophilized dosage form as a commercial product. The objective of these studies was to devise a stable, rapid-dissolving, therapeutically compatible dosage form for this compound.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade except when noted. (R,R)-formoterol (L)-tartrate was supplied by Sepracor, Inc.; mannitol USP was obtained from ICI America, and water for injection was obtained from Abbott Laboratories.

Subambient DSC Studies

Differential scanning calorimetry (DSC) was used to determine the glass transitions of frozen formoterol formulations. Glass transition temperatures were measured in frozen solutions using a computer-aided Perkin-Elmer DSC 7 differential scanning calorimeter. A mechanical cooling accessory was used for cooling the sample chamber to temperatures as low as -40°C. Sample solution (20 μL) was placed in an aluminum sample pan. An aluminum top was placed on the sample pan and crimped in place, but not hermetically sealed. The sample solutions contain 0.8% of formoterol and 10% lactose or 10% mannitol as a diluent. The sample solution was cooled from 25 to -40°C at a rate of 5°C/min and held at -40°C for 5 min before heating to ensure thermal equilibration. The sample was then heated from -40 to 25°C at 10°C/min. Thermograms were recorded for both the cooling and heating cycles.

X-Ray Powder Diffraction Characterization

Qualitative x-ray powder diffraction analysis was carried out using a PW 1710 diffractometer (40 kV, 30 mA, Philips Electronic Instruments, Mount Vernon, NY) with PC-APD v2.2b diffraction software. Graphite monochromatized copper Kα radiation was used. An approximate 500-mg sample was placed in an aluminum sample holder using a back-filling method to reduce crystal orientation effects. The diffraction patterns were collected over the range 5 to 65° 2θ at a rate of 2.5° 2θ per min.

HPLC Analysis

The column was Zorbax SB-CN (4.6 mm $\times$ 25 cm), and the mobile phase was composed of 25% acetonitrile and 75% aqueous 0.05 M phosphate buffer with 0.01 M hexanesulfonic acid (pH 3.0). Analytical wavelength was 220 nm, and the flow rate was 1 mL/min. The major degradation product, desformoterol, was well separated from the main peak.

Electrochemical Methods

For Karl Fisher moisture determinations, glassware was oven-dried for at least 2 hr and stored in a desiccator before use. The lyophilized product was dissolved in the vial with 1.0 mL of formamide. The solubilized product (0.8 mL) was removed and injected into a 684 KF coulometer (Metrohm). Titrations were performed, and the product moisture was calculated as a percent of product weight.

pH measurements were made using two standard points on an Accumet Model 25 pH meter (Fisher Scientific).

Solution Stability Screening

The solutions of approximately 1 mg of formoterol in 5 mL of 0.1N HCl and 0.1 N NaOH were incubated at 60°C for 22 hr. The degradation of the sample was then measured using HPLC.

The stability of aqueous formoterol and aqueous formoterol (0.050 mg/mL) in the presence of 0.050 mg/mL

acetate and/or 10.0 mg/mL of α-lactose at 50°C was evaluated at 50°C for 7 days. One neat solution of formoterol was studied without pH adjustment; for all other solutions the initial pH level was adjusted to 4.81 ($\pm$0.03) with dilute sodium hydroxide.

pKa Determinations

The dissociation constant for the phenolic hydroxyl group was determined spectrophotometrically (5) by preparing 0.0005 M formoterol tartrate solutions in the pH range of 5 to 13 using acetate, phosphate, borate, and sodium hydroxide buffers. In general, the buffer concentrations were approximately 0.1 M. No pH changes were observed on addition of the drug to buffer solutions. The absorbance spectra (200 to 400 nm) were collected on a Shimadzu UV-2101 PC spectrophotometer. These spectra were unchanged for at least 0.5 hr, and thus the absorbance values were used without corrections for possible drug instability.

Formulation Development

Test formulations were prepared in accordance with the manufacturing flow chart depicted in Figure 1. Buffer excipients were dissolved in approximately 80 mL of double distilled water (DDW), then adjusted to an appropriate pH, and finally diluted to 100 mL with DDW. An appropriate amount of formoterol was accurately weighed to give a final concentration of 0.005% (w/v), and appropriate amounts of excipients were added. Solids were dissolved in DDW with stirring at ambient temperature. On complete dissolution, the solution was adjusted to the final volume with DDW. The pH was checked.

The solution was filtered through a MilliPak-20, 0.22-μm filter peristaltic pump (Master Flex, model 7518-62). Silastic tubing (0.188 in. i.d. $\times$ 0.375 in. o.d.) was used upstream from the filter. The pump speed was adjusted so that filtration took place at a slow to moderate rate. The solution was transferred to a Pyrex container at ambient

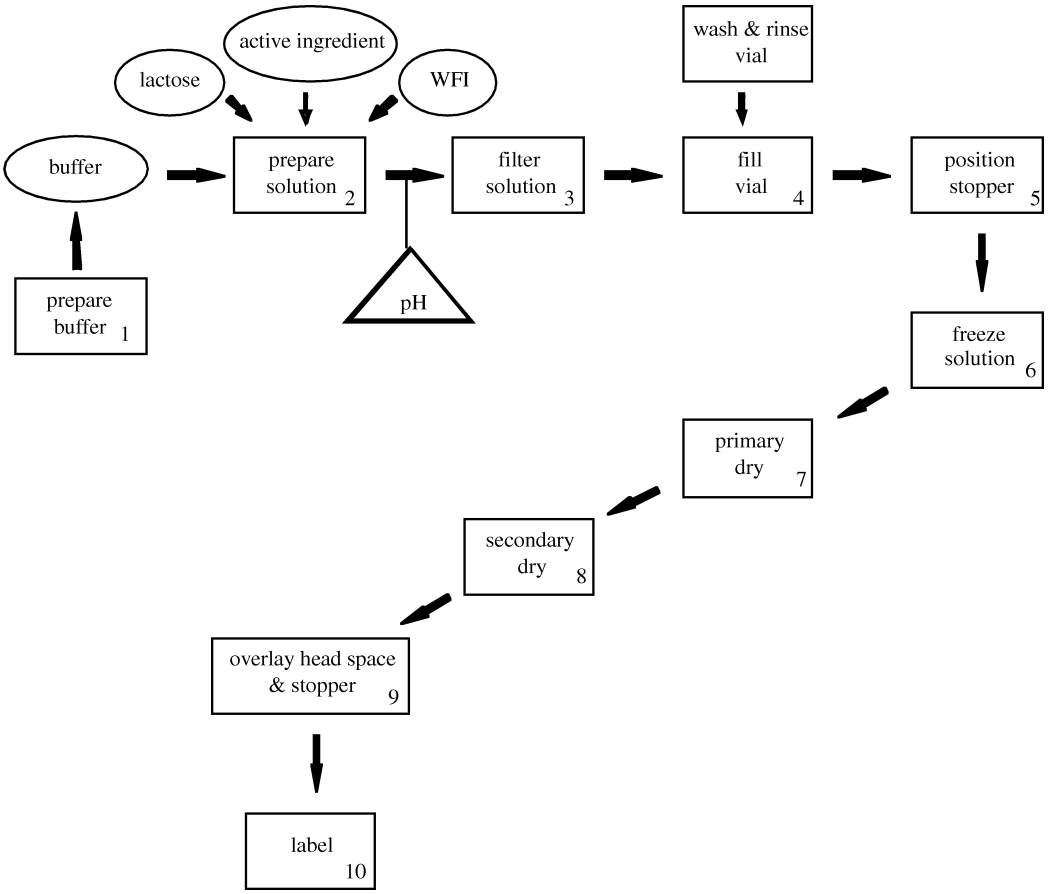

Figure 1. Process flow diagram for the preparation of lyophilized (R,R)-formoterol lots.

temperature. The filtered solution (1.0 mL) was filled into type 1 glass vials using a calibrated autopipette. Butyl rubber lyo stoppers were positioned on each vial.

Two vials with thermocouples were prepared, and all of the vials were loaded into a freeze dryer (Advantage, Virtis) at ambient temperature. Shelf temperatureva was lowered to −50°C, and product was allowed to freeze at this temperature using a 1-hr hold time.

The condenser temperature was allowed to reach −50°C, and the chamber was evacuated to ≤100 μm. Primary drying was conducted at −40°C for 17 hr. Secondary drying occured at −28°C for 17 hr, followed by 0°C for 9 to 17 hr and at 25°C for 4 to 17 hr. After secondary drying, the vacuum was neutralized with air, and stoppers were seated using the freeze-dryer stoppering mode.

After lyophilization, formulations were characterized by residual moisture, HPLC potency and pH (Table 1).

Stability Studies

Formulation assessments were carried out by subjecting lyophilized products to combinations of thermal and moisture (0 to 90% relative humidity) stress. HPLC potency, pH level, and residual moisture were measured periodically to evaluate product stability.

Table 1.

Summary Results of (R,R)-Formoterol (0.05 mg/Vial) Lot Characterization

Lot	Excipient	Mg/Vial	Residual Moisture %	Potency Mean mg/mL	Mean pH
A	Lactose	10.0	1.10	49.3	5.29
	Acetate	0.050			
B	Mannitol	10.0	0.91	49.5	5.40
	Acetate	0.050			
C	Lactose	0.0	332	48.0	5.18
	Acetate	0.0			
D	Lactose	0.0	15.8	48.3	6.80
	Acetate	1.0			
E	Lactose	10.0	1.88	49.2	5.38
	Acetate	0.0			
F	Lactose	10.0	1.68	49.8	5.09
	Acetate	1.0			
G	Lactose	1.0	8.33	50.2	4.54
H	Lactose	5.0	3.21	48.8	4.50
I	Lactose	10.0	2.94	46.0	4.76
J	Lactose	50.0	1.22	47.4	4.92

Table 2.

The Composition of the Humidity Chambers and the Measured Relative Humidity at 25, 40, and 50°C

Composition	% RH: 25°C	40°C	50°C
drierite/P_2O_5	0		0
LiCl	11.3		10.9
CH_3COOK	24.5	17.7	14.6
$MgBr_2$	36.4	33.0	29.5
MgN_2O_6	52.8		51.0
$Na_2Cr_2O_7$	64.4	57.2	49.7
NaCl	91.8	89.3	88.7

Stress conditions were achieved by placing open vials containing various formulations into humidity chambers and placing the humidity chambers in 50, 40, or 25°C environmental chambers. Various levels of moisture exposure were obtained by using saturated salt solutions as described in Table 2. The relative humidities were determined at 50, 40, or 25°C. HPLC, pH level, and residual moisture assays were conducted as described previously.

The stability of the formulation was quantified by determining the initial degradation rate (usually <20% degradation).

RESULTS AND DISCUSSION

Subambient DSC Studies

On cooling, both formoterol-lactose and formoterol-mannitol solutions show a strong exothermic peak. The formoterol-lactose and formoterol-mannitol solutions completely froze at −24.3 and −29.5°C, respectively. Furthermore, the formoterol-mannitol solution had a small exothermic peak before the main exotherm at approximately −14°C.

On heating, the formoterol-lactose preparation showed a strong endotherm characteristic of a melting transition at −2.7°C. A change in enthalpy was found at approximately 35°C. Based on this observation, primary drying was conducted at −40°C. Seventeen-hour drying intervals were selected based on thermocouple and chamber drying indicator observations during trial lyophilizations.

On heating, the formoterol-mannitol preparations exhibited a stong melting transition at −0.9°C. The glass transition temperature of formoterol-mannitol solutions was approximately −22°C. For ease of preparation, mannitol formulations were lyophlized using the same cycles as for the lactose formulations.

Solution Stability Screening

The pH of unbuffered formoterol (without initial pH adjustment) increased 0.4 pH U with a concomitant 7.9% loss of formoterol in 3 days (50°C) and 9.1% in 7 days. The pH-adjusted, unbuffered formoterol solution experienced a 1.3 U pH increase and a 5.5% drug loss over the same time, but at 7 days the pH was constant and the drug loss increased to 18.1%. The pH was constant, and a 3.5% drug loss in 7 days was observed in formoterol solutions containing only acetate. The results for the acetate/lactose/formoterol combination were similar (0.1 pH U increase and 2.6% drug loss in 7 days). No significant pH change or drug loss was observed in the solution composed of lactose and formoterol without acetate buffer.

pKa Determinations

The wavelengths of maximum pH-dependent absorbance changes were 250, 282, and 311 nm. The dissociation constant was obtained using absorbance data at each wavelength by fitting to the following equation using the nonlinear regression method in JMP (SAS software).

$$Absorbance_{\lambda_{nm}} = (\varepsilon_{FH} \times f_{FH} + \varepsilon_F \times f_F) \times C_{total}$$

where

$$f_{FH} = \frac{10^{-pH}}{(10^{-pH} + K_1)}$$

$$f_F = \frac{K_1}{(10^{-pH} + K_1)}$$

and ε_{FH} and ε_F are the molar absorbtivities at each analytical wavelength, which were estimated by averaging the absorbance values at pH values 5 through 7 and 11 through 13, respectively, and dividing the averages by the total molar concentration of formoterol (C_{total}). The results of the nonlinear regression are shown in Figure 2.

The values of the dissociation constant, K_1, were 3.24×10^{-9}, 4.68×10^{-9}, and 2.40×10^{-9} at 250, 282, and 311 nm, respectively. Thus the average dissociation constant was 3.44×10^{-9}, and the pKa is 8.5.

Initial Characterizations of Formulations

Lactose formulations had residual moistures between 1 to 2% (Table 1). XRD showed the absence of crystallinity. Mannitol formulations dried rapidly (lot B, Table 1). Residual moistures were approximately 1%. XRD showed significant crystal structure in the lyophilized mannitol product.

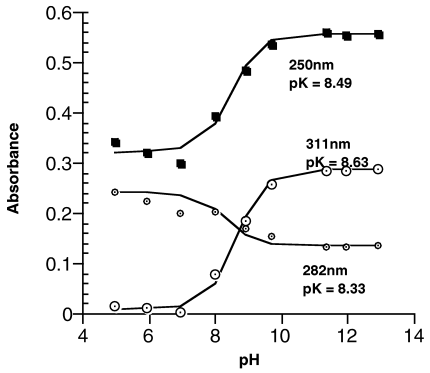

Figure 2. pKa determination for aqueous (R,R)-formoterol at 25°C by spectrophotometric analysis.

A significant increase in pH was observed during lyophilization in the formulation that contained only drug and acetate buffer (lot D, Table 1). This was likely attributable to the vaporization of acetic acid during primary and secondary drying.

Potencies were generally within 2% of target values (Table 1).

Formulation Stability Assessments

The residual moisture content varied by formulation and relative humidity (Fig. 3). The moisture content of mannitol/formoterol formulation (lot B) was ≤1% at all humidity levels. This result is typical of mannitol

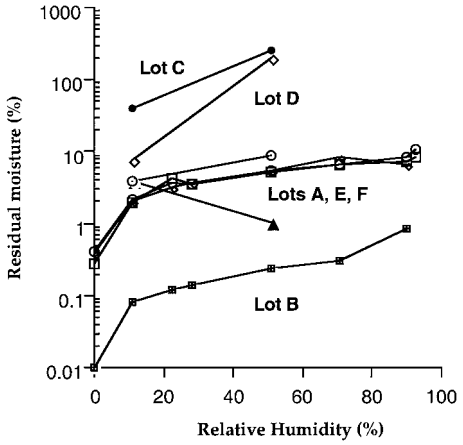

Figure 3. Residual moisture content (determined by Karl Fisher method) of various (R,R)-formoterol formulations stored at 50°C at a function of relative humidity.

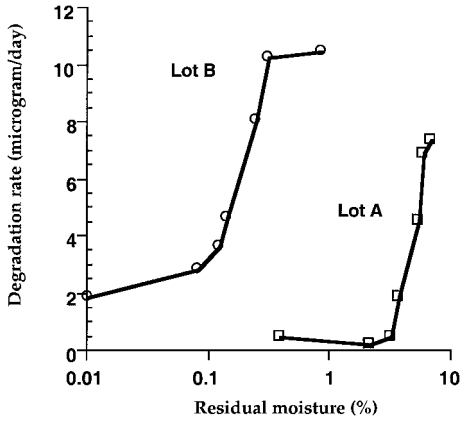

Figure 4. (R,R)-formoterol degradation rate at 50°C as a function of residual moisture for formulations containing 10 mg of mannitol (lot B) or lactose (lot A) and 50 mcg of sodium acetate.

formulations wherein the mannitol crystallizes during lyophilization. The extremely high residual moisture for formulations with low total solids content (lots C and D) may be attributable to difficulties in sampling.

The initial degradation rate was strongly dependent on the residual moisture. For example, the degradation increased from essentially 0 to 4 mcg/mL per day as the residual moisture increased from 2 to 4% for lot A (Fig. 4). The critical transitional moisture content (at 50°C) for this formulation appeared to be approximately 3.2%.

The effect of mannitol on formoterol degradation was profound. In Figure 4, the degradation of formoterol in mannitol (lot B) was significantly greater than that in lactose formulations (lot A) despite the much lower residual moisture content of mannitol-based formulations. This may be attributed to the intimate contact of drug and moisture on the surface of crystalline mannitol. Thus, although the total residual moisture for these formulations is low, the effective moisture content in the vicinity of the drug is high.

A study was conducted to confirm that formoterol degradation was primarily attributable to hydrolysis rather than to oxidation. The degradation of lyophilized mannitol/formoterol and lactose/acetate/formoterol formulations and bulk crystalline formoterol with and without lactose were subjected to thermal stress (50°C) under either a nitrogen or air headspace. Although the mannitol-based formulation was relatively unstable, no differences were observed between any samples stored under nitrogen and air. Thus oxidative degradation does not appear to play a significant role in formoterol stability.

A factorial study was conducted to determine the effects of acetate and lactose on lyophilized formoterol

Table 3.

Design and Results of a Factorial Study to Determine the Effect of Lactose and Acetate Excipient on Solid-State (R,R)-Formoterol Stability at 50°C

Lot (4108-WM-)	Acetate (mg/Vial)	Lactose (mg/Vial)	RH %	Degradation Rate (mcg/day)
C	0	0	11	0.00
	0	0	51	−0.29
D	1	0	11	−1.36
	1	0	51	−5.60
E	0	10	11	0.00
	0	10	51	−0.64
F	1	10	11	−0.36
	1	10	51	−5.40

stability (lots C through F). Degradation was measured by time-dependent formoterol loss. Formulations were stored at low (11%) and high (51%) relative humidities (Table 3).

The effects of formulation components on the degradation rates were analyzed using the following regression model and JMP software (SAS Institute).

$$Degradation\_rate = \beta_0 + \beta_1[acetate] + \beta_2[lactose] + \beta_3 RH + \beta_{12}[acetate][lactose] + \beta_{13}[acetate]RH + \beta_{23}[lactose]RH$$

The summaries of the analyses are shown in Table 4. The only significant effect of the tested variables on the degradation rate was attributed to an acetate/relative humidity interaction. At high relative humidity, the presence of acetate significantly increased the degradation rate in the presence or absence of lactose. These results suggest that a low residual moisture, lactose/formoterol formulations without acetate may be sufficiently stable.

The optimum lactose level was determined by evaluating four lots of formoterol containing 1, 5, 10, or 50 mg of lactose (lots G through J) and subjecting them to thermal stress (25, 40, or 50°C) at various moisture levels (0 to 92% relative humidity (Table 2). Time-dependent potency changes were monitored. Residual moisture and pH were also determined periodically. Initial rates of potency loss were calculated and compared with the moisture stress and lactose level at each temperature using contour plots (6).

In general, formulations containing $\geq$10 mg of lactose were less labile under all thermal conditions (shaded areas in Fig. 5). At 50°C, the stabilizing effects of lactose at low relative humidity were not as evident as at 40 and 25°C. The 40°C results indicated that the optimum lactose level was >30 mg and the residual moisture should be <3%.

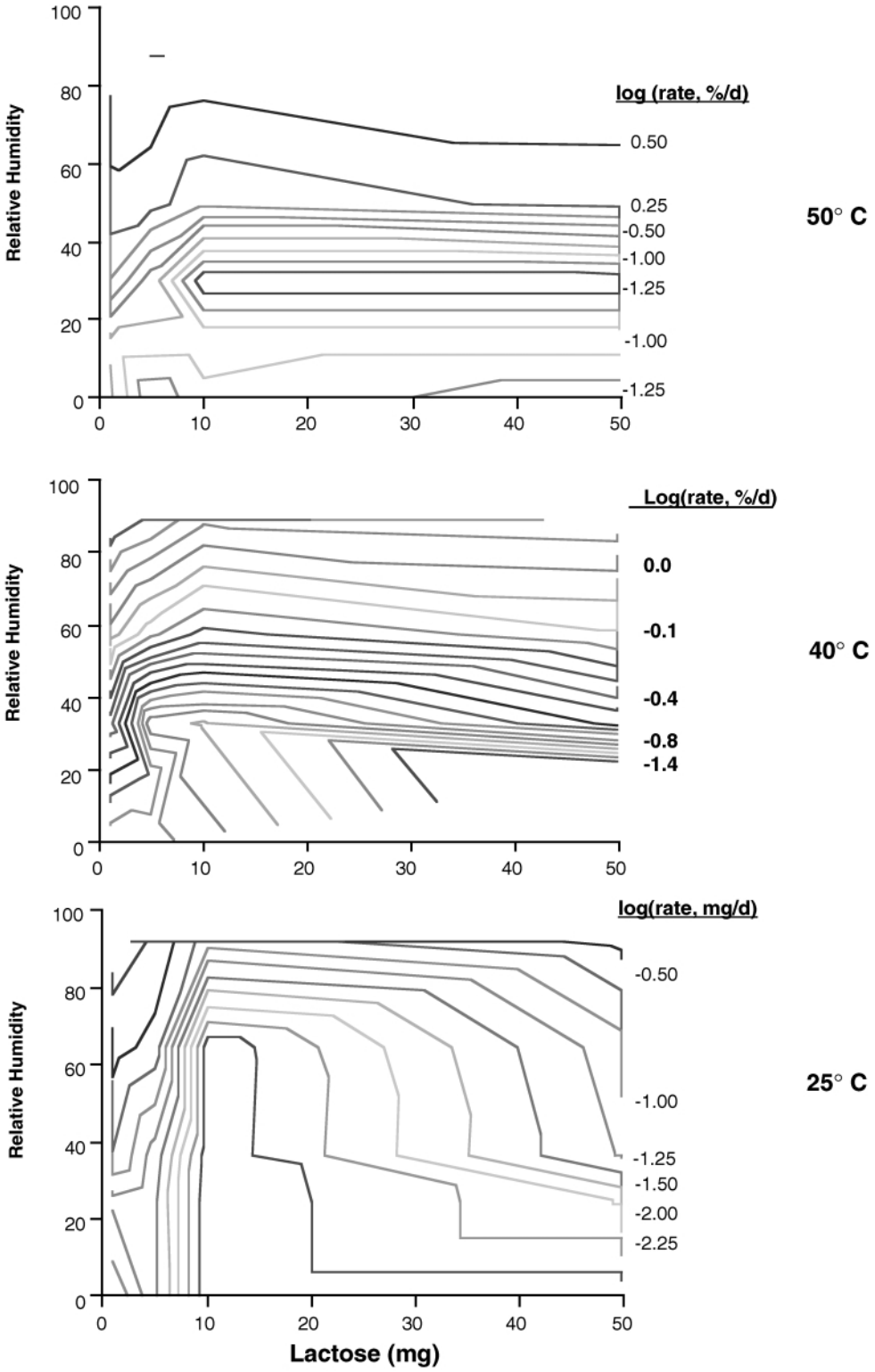

Figure 5. Contour plots depicting the effects of relative humidity and lactose content on the degradation rate of lyophilized (R,R)-formoterol at 25, 40, and 50°C.

Table 4.

Summary of Regression Model Fit for (R,R)-Formoterol Degradation Rate Data

Effect Test					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
acetate	1	1	1.0375042	40.9878	0.0986
lactose	1	1	0.0084375	0.3333	0.6667
RH	1	1	0.0210042	0.8298	0.5297
acetate ∗ lactose	1	1	0.3003125	11.8642	0.1799
acetate ∗ RH	1	1	8.7153125	344.3086	0.0343
RH ∗ lactose	1	1	0.1653125	6.5309	0.2375

Note: Independent variables were treated as ordinal (categorical) data.
Rsquare, 0.999362; rsquare adj, 0.995537; root mean square error, 0.159099; mean of response, 1.70625; observations (or sum wgts), 8.

The 25°C results indicated that a broader range of lactose levels was acceptable; however, data were limited owing to the time interval needed to observe significant degradation at this temperature. For the formulation containing 50 mg of lactose, no degradation was observed in samples stored up to 48 and 62 days at 50 and 40°C, respectively.

CONCLUSIONS

The development of lyophilized formoterol formulations was undertaken to ensure an adequate product shelf-life. Solid-state instability was observed as a function of high residual moisture and diluents. Mannitol was shown to be a unsuitable excipient. The presence of acetate salts and high moisture was found to be problematic as well. Based on these results and the need for a diluent in vials containing microgram quantities of drug, lactose was selected. The level of lactose was optimized by evaluating the degradation rate as a function of lactose content and relative humidity over a range of storage temperatures. At low humidity, 50 mg of lactose resulted in optimal stability over the temperature range studied.

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