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L-649,923 – The selection of an appropriate salt form and preparation of a stable oral formulation

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Abstract

L-649,923 is an orally active leukotriene D_4 antagonist and the salt of a γ-hydroxy acid. It was formulated as a physically and chemically stable compressed tablet dosage form. Because the free acid was inherently unstable, the physical and chemical properties of the sodium, calcium, ethylenediamine and benzathine salts were evaluated and the calcium salt selected as the most pharmaceutically acceptable form of the drug for formulation development. The principal route of degradation was through an intramolecular esterification to yield the γ-lactone. Degradation could be monitored by following the appearance of the γ-lactone with an HPLC assay. A mechanism of degradation was proposed resembling acid-catalyzed solution esterification occurring in the sorbed moisture. This postulate was used as a predictive model. Formation of γ-lactone was minimized in a direct compression tablet formulation by reducing the amount of free acid in the drug substance, avoiding an aqueous granulating process, using excipients with low water content and adding sodium carbonate as an alkalizing agent.

Key words: L-649,923; Leukotriene D_4 antagonist; Formulation stability; Drug degradation; Salt form selection; Degradation mechanism; Predictive model

1. Introduction

L-649,923 is an orally active, selective receptor antagonist of leukotriene D_4 (LTD_4). As part of a screening process with a general class of substituted phenylketobutyric acids, the compound was synthesized (Young et al., 1986a) and shown to selectively antagonize LTD_4 in in vitro and in vivo models (Jones et al., 1986, 1988; Young et al., 1986b, 1987). The background and rationale for the selection process are described elsewhere (Young et al., 1986b; Young, 1988). LTD_4 is a very potent bronchoconstrictor (Zakrzewski et al., 1985) implicated in the pathophysiology of asthma. A receptor antagonist may alter the onset and development of respiratory disease. L-649,923, tested in the clinic, was shown to weakly antagonize LTD_4 (Barnes et al., 1987). It caused small but significant changes in early response pulmonary function with antigen-induced (Britton et al., 1987a,b) and LTD_4-induced bronchoconstriction (Barnes et al., 1987, 1988).

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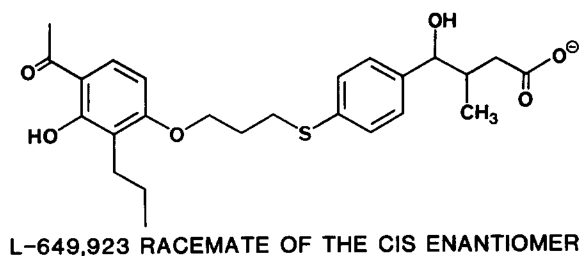

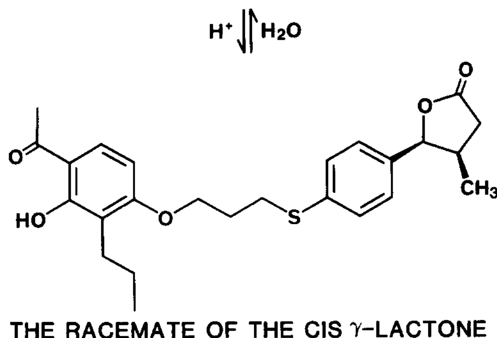

Fig. 1. The conjugate base of the L-649,923 *cis* γ-hydroxy free acid and its *cis* γ-lactone. The compound consists of *cis* and *trans* diastereoisomers of which L-649,923 is a racemate of the two enantiomeric pairs of the *cis* diastereoisomer. The corresponding γ-lactone which forms is the *cis* γ-lactone.

The compound contains two asymmetric centres (Slobodzian et al., 1987). The diastereoisomers consisting of the enantiomeric pairs ($\beta R^*,\gamma R^*$), ($\beta S^*,\gamma S^*$) and ($\beta R^*,\gamma S^*$), ($\gamma S^*, \beta R^*$) were prepared from the *trans* and *cis* γ-lactone, respectively (Young et al., 1986a). The *trans* diastereoisomer was less active in vivo. The two entaniomers of the *cis* diastereoisomer appeared to have comparable activity (Young et al., 1986a). Rather than develop an enantiomerically pure form, the racemate, derived from the *cis* γ-lactone, was chosen for drug development.

The free acid of the racemate was difficult to isolate as pure, stable material because the free acid form equilibrated rapidly with the less active cyclic γ-lactone in solution (see Fig. 1) (Young et al., 1986a). To obtain the open hydrolyzed form of the drug with satisfactory purity and yield, it was critical to isolate the compound as a salt form. Therefore, the primary objective was to select a salt form of the racemate which was physically and chemically stable in a conventional oral solid dosage form, preferrably a compressed tablet. A variety of salts were evaluated to select a form of the drug with pharmaceutically acceptable physical properties. Four salt forms with cationic counterions (Berge et al., 1977) were prepared successfully in sufficient quantities for evaluation. Selection of the appropriate form of the drug permitted the development of a stable formulation.

2. Materials and methods

2.1. Materials

L-649,923 salts

The salt forms of L-649,923 were prepared using the methods reported previously (Young et al., 1986a,b). The benzathine salt was obtained from an aqueous solution of the sodium salt. The calcium and ethylenediamine salts were obtained from hydrolysis of the γ-lactone.

Excipients

The following excipients were used in excipient compatibility studies and in the preparation of solid dosage forms: microcrystalline cellulose, N.F. from FMC Corp. (Avicel PH 101, 103); spray-dried lactose, N.F. from Formost Whey Products; pregelatinized starch (Starch 1500), N.F. from Colorcon, West Point, PA; croscarmellose sodium, N.F. (Ac-Di-Sol) from FMC Corp.; magnesium stearate, N.F. from Witco, Montreal; hard gelatin capsules from Capsugel; sodium carbonate, glycine and sodium phosphate from Baker.

2.2. Methods

X-ray powder diffractometry (XRPD)

Diffraction patterns were obtained using a Philips X-Ray Powder Diffractometer (Philips PW 1840) with copper Kα radiation (1.5418). Response was measured as a function of the 2θ angle.

High-performance liquid chromatography (HPLC)

An HPLC stability-indicating assay method was developed to monitor degradation of the drug

substance and pharmaceutical preparations. Hewlett Packard Model 1084B HPLC instrumentation was employed with a variable wavelength UV detector. An Altex Ultrasphere column (150 mm × 4.6 mm i.d., 5 μm packing material) was used at 40°C with a mobile phase flow rate of 2.0 ml/min. The mobile phase was 70% v/v methanol in 0.025 M aqueous sodium phosphate buffer (pH 3.0). The detection wavelength was 290 nm. The sample was dissolved in HPLC grade DMF (1 mg/ml) and 15 μl were injected. Retention times for the parent compound and its *cis* γ-lactone were approx. 7.5 and 13 min, respectively. The γ-lactone present in samples of drug substance and dosage forms was determined as an area percent from HPLC chromatograms.

Thermal stability

Thermal stability was assessed by placing samples in amber glass screw cap vials in temperature-controlled ovens for specific time periods. The samples were removed from the oven at the end of the time period and analyzed by the HPLC stability-indicating assay method.

Photosensitivity

Photochemical stability was evaluated (4 klux, 350 footcandles) in a fluorescent light cabinet. Samples were placed in open containers in the light cabinet for 6 weeks. Average cabinet temperature was 30°C. The samples were removed and assayed using the HPLC stability-indicating assay.

Hygroscopicity

The hygroscopicity was assessed by determining the amount of water uptake after sample equilibration in constant relative humidity chambers. Samples, dried over phosphorus pentoxide for at least 48 h, were weighed using a Mettler analytical balance and placed in open vials in constant relative humidity chambers for 72 h. Samples were removed and immediately reweighed. Weight gain was determined from the change in sample weight and water uptake determined as a percent of the initial weight.

Solubility

The solubility in aqueous solutions was determined by adding sufficient salt to the aqueous solvent in stoppered vials until a significant quantity of solid material was visible in the bottom of the vial. The vials were sonicated for at least 24 h to obtain a saturated solution. Vials were removed from the sonicator and allowed to equilibrate under ambient conditions for several hours. Samples were centrifuged and the supernatant removed by pipette. The drug concentration in the supernatant was determined spectrophotometrically at 290 nm.

Drug solutions were stable for the duration of the studies. Solutions of the sodium salt prepared at concentrations of 2, 10 and 30 mg/ml were stored at room temperature for 48 h. Analysis by HPLC indicated loss of intact drug of < 2%. The primary degradation product was the γ-lactone with a similar chromophoric response to that of the drug.

Intrinsic dissolution

Intrinsic dissolution was determined using a method similar to that described elsewhere (Hanson, 1991). Drug substance was compressed into a stainless-steel die to obtain a flat circular disc of solid material with one exposed surface. The exposed surface area was 0.385 cm^2. The die with the compressed disc was clipped to the bottom of a USP Apparatus 1 dissolution basket drive rod. This assembly was installed in the USP dissolution apparatus. The apparatus was operated at 100 rpm with 200 ml of dissolution medium (Tris buffer, pH 7.2, ionic strength 0.1). Samples (1 ml) were taken every 2 min for 20 min and analyzed for drug concentration by UV spectrophotometry. The intrinsic dissolution was determined at individual time points and a mean determined from the final equilibrium values.

Determination of pK_a

The pK_a was obtained by determining the solubility of the salt in aqueous solutions buffered at various pH values employing the method outlined elsewhere (Zimmerman, 1983; Albert et al., 1984). Buffers were prepared according to empirical buffer tables at a constant ionic strength of

0.1. Final pH values were measured with a pH meter.

Drug-excipient compatibility

Samples were prepared by grinding the drug substance and excipient with mortar and pestle using the following drug/excipient ratios (w/w): microcrystalline cellulose, 1:3; spray-dried lactose, 1:3; pregelatinized starch, 1:1; croscarmellose sodium, 1:1; magnesium stearate, 1:1. Prepared samples were stored in screw cap vials. Vials were placed in temperature-controlled ovens under accelerated thermal stability conditions at 50 and 80°C and removed at appropriate time intervals. Samples were analyzed by HPLC.

Preparation of trial capsule formulations

An initial capsule formulation was prepared using an aqueous granulation process. Drug substance was granulated with pregelatinized starch. Magnesium stearate was the lubricant. The granules were dried overnight at 35-40°C in a convective air dryer. They were screened through a 30 mesh sieve and encapsulated in No. 0 hard gelatin capsules.

In a parallel series of experiments, a dry powder blend of capsule fill was prepared. The drug substance was dry blended with microcrystalline cellulose (Avicel PH101), screened through a 60 mesh sieve and encapsulated in a No. 0 hard gelatin capsule. The potency, content uniformity, dissolution and chemical stability were evaluated by HPLC.

Preparation of trial tablet formulations

Small laboratory batches (30-50 tablets) were prepared at two dose strengths (250 and 375 mg). The ingredients were dry mixed in a mortar with pestle followed by tumble mixing for at least 10 min. The granulation was compressed on a single punch tablet press. For the 250 mg direct compression dose strength, total tablet weight was 450 mg and hardness was 7-8 kp. Each tablet contained 164.4 mg microcrystalline cellulose (Avicel PH103), 27.7 mg sodium carbonate (equimolar), 4.5 mg croscarmellose sodium (1%) as a disintegrant and 3.4 mg magnesium stearate (0.75%) as the lubricant. The 375 mg dose strength tablets were prepared in identical fashion using the same drug/excipient ratios.

3. Results and discussion

3.1. Crystallinity

Of the four salt forms isolated, the sodium and calcium salts were amorphous material by powder X-ray diffractometry. They were never successfully crystallized. The ethylenediamine and *N,N'*-dibenzylethylenediamine (benzathine) salts were crystalline by X-ray diffractometry. The ethylenediamine salt was isolated as thin plate-like crystals. The benzathine salt was obtained as long acicular crystals.

3.2. Thermal stability

The short-term accelerated thermal stability data obtained for the sodium salt, employing the stability-indicating HPLC assay method, indicated significant loss of intact drug which appeared to correlate well with formation of the *cis* γ-lactone. A typical HPLC chromatogram is shown in Fig. 2. From the data obtained, it appeared that the formation of the γ-lactone, with a similar UV chromophoric response to that of the parent compound, was a good monitor of drug degradation. Examination of the data for the calcium salt at 80°C clearly demonstrated that, after 3 months, the primary degradation product was the γ-lactone (Fig. 3). A good material accountability was obtained from the area percent of the peaks corresponding to the parent compound and its γ-lactone. Data from samples of the sodium salt stressed at 60°C/2 weeks and at 80°C/1 week indicated that isomerization to the *trans* γ-lactone had occurred under these conditions. However, the *trans* γ-lactone was not detectable in stressed samples of the sodium salt at temperatures below 60°C nor was it present in stressed samples of the calcium salt at temperatures of 80°C or less. The *cis* diastereoisomer, L-649,923, prepared as pilot batches of the calcium salt, contained less than 0.5% of its corresponding *trans* isomer. No evidence for *cis-trans*

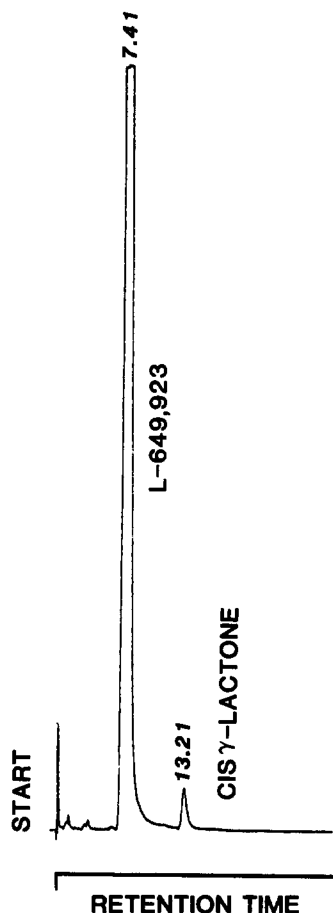

Fig. 2. An HPLC chromatogram of L-649,923 with its associated γ-lactone degradation product eluting as a minor peak at 13.2 min. HPLC stability-indicating assay conditions are described in the text.

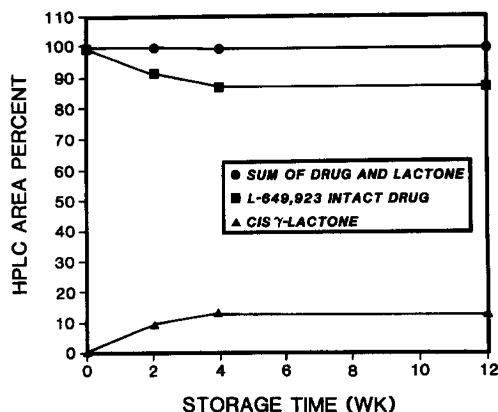

Fig. 3. Data obtained using the HPLC stability-indicating assay which demonstrates good material accountability when the calcium salt is degraded at 80°C for 3 months.

isomerization of the calcium salt was observed under the conditions of accelerated thermal stress. A comparison of drug stability for the sodium, calcium and benzathine salts at three temperatures is shown in Table 1.

Samples of the ethylenediamine salt stressed at 40°C/2 weeks contained approx. 80% intact drug. Results from these thermal stability studies indicated that the ethylenediamine salt lacked the desired solid-state thermal stability for drug development.

The crystalline benzathine salt appeared to have the best stability with the amorphous calcium salt a second possibility. A separate study with the benzathine and calcium salt forms cor-

Table 1
Comparative stability data for the sodium, calcium and benzathine salts

Time (weeks)	γ-Lactone formed (area percent)								
	Sodium salt			Calcium salt			Benzathine salt		
	30°C	40°C	50°C	30°C	40°C	50°C	30°C	40°C	50°C
2	–	3.0	6.9	0.4	2.0	2.6	0	0	0.4
4	2.1	4.8	–	0.6	1.9	3.0	0	0.3	0.5
6	2.4	6.2	–	0.7	2.0	3.2	0	0.4	0.8
8	2.8	6.3	–	0.8	2.4	3.0	0.1	0.2	0.7

The formation of γ-lactone as an indication of drug degradation expressed as an HPLC area percent (corrected for γ-lactone observed in the assay of the initial samples) is tabulated as a function of thermally stressed storage times for the temperature conditions 30, 40 and 50°C.

roborated this conclusion. Neat dry material of each salt was stressed at 50°C/4 weeks in screw cap vials. From this study, samples of stressed benzathine salt contained 0.4% γ-lactone. Samples of stressed calcium salt contained 3.8% γ-lactone.

3.3. Photosensitivity

Data were obtained from a limited stability study with samples exposed to 4 klux of fluorescent light for 6 weeks. Results, expressed as a percentage of intact drug remaining in the sample, were: sodium salt, 98%; calcium salt, 98%; benzathine salt, 99%. No significant amount of light-induced degradation was expected under ambient conditions. The ethylenediamine salt with its unsatisfactory thermal stability was not evaluated in this study.

3.4. Hygroscopicity

The amount of water sorbed at 76% relative humidity was determined for the four salt forms. The ethylenediamine and benzathine crystalline salts were nonhygroscopic sorbing < 0.1 and 0.2 wt%, respectively. The amorphous calcium salt was somewhat hygroscopic sorbing 1.2% water. The sodium salt was very hygroscopic sorbing 18 wt%.

The equilibrium water uptake as a function of relative humidity for the sodium salt is listed in Table 2. At a relative humidity of 35% or greater, the appearance of the material changed from a white powder to a tacky gum. This phenomenon has been observed for other candidates in this same class of compounds, notably L-660,711 (Vadas et al., 1991). The change in physical properties of these materials with water uptake hinders their formulation as a solid oral dosage form. It is preferable to use a salt form which does not undergo change in its solid-state properties when exposed to high relative humidities.

Table 2
Sodium salt hygroscopicity

Relative humidity (%)	Weight gain of sodium salt (%)
15	0.5
20	1.3
30	2.1
35	4.1
47	6.4
66	13
76	18

Water uptake is expressed as percent weight gain of total sample weight. Samples were equilibrated initially over phosphorus pentoxide for at least 48 h, then stored in relative humidity chambers for at least 72 h.

3.5. Solubility

The equilibrium solubilities of the four salts were determined in water. The data indicate that the sodium salt was freely soluble (> 300 mg/ml) whereas the ethylenediamine (< 0.1%), calcium (0.2 mg/ml) and benzathine (0.1 mg/ml) salts had lower solubilities. The pH of the saturated aqueous solutions of the sodium salt was 7.8–8.0. Saturated solutions of the calcium salt had pH values of 7.2. Although pH will affect equilibrium solubilities, the small differences in pH of the saturated solutions cannot account for the majority of the observed differences in solubility.

Equilibrium solubilities of the calcium salt in aqueous buffers were evaluated as a function of pH (Table 3). From these data an apparent pK_a of 5.6 ± 0.4 at 0.1 ionic strength was calculated.

Table 3
Equilibrium aqueous solubilities for the calcium salt determined at room temperature in buffered solutions at 0.1 ionic strength and various pH values

Buffer	pH	Amount dissolved (mg/100 ml)	Calculated pK_a
0.1 N HCl	1.03	0.003	–
Acetate	5.09	0.004	5.57
Phosphate	6.20	0.011	5.77
Phosphate	6.22	0.010	5.85
Phosphate	7.03	0.27	5.08
Tris-HCl	8.01	1.88	5.21
Tris-HCl	8.04	2.03	5.21
Tris-HCl	8.99	2.60	6.05
Tris-HCl	9.02	3.44	5.96

From these data the pK_a was calculated at each pH value. The mean value was 5.6 ± 0.4.

No significant buffer or common ion effects were observed.

3.6. Selection of the salt form

The ethylenediamine salt was not suitable because of the considerable degradation observed at 40°C. The sodium salt is the most frequently used salt form in pharmaceutical preparations (Berge et al., 1977). However, in this case, its hygroscopicity with the associated changes in physical properties was undesirable. It was not as thermally stable as either the calcium or the benzathine salts. The latter two salts were considered the best options.

The calcium salt was amorphous. Developing a stable pharmaceutical formulation with amorphous material which may subsequently crystallize is an undesirable risk (Mullins et al., 1960). Amorphous materials generally are less stable chemically than those in the crystalline state. They are often difficult to characterize and result in greater batch-to-batch variations. However, the calcium salt had not been crystallized despite several attempts. The difficulty experienced crystallizing the compound may be because the salt, containing two organic carboxylate anions of a racemate, could not easily form the required constrained crystal lattice network during the nucleation process. As an amorphous material it appeared to have good physical stability. From the experience obtained during preparation of laboratory-scale batches of material, batch-to-batch reproducibility did not appear to be a problem. If the calcium salt was used, the development of a physically stable solid dosage form appeared likely.

The benzathine salt was crystalline with very good chemical stability. It appeared to be the salt form of first choice. However, its lower solubility and possible lower bioavailability when compared with the calcium salt were a concern. A preliminary comparative study in the rat indicated that the benzathine salt may not be as bioavailable as the calcium salt. In this case relative bioavailability was assessed by comparing the plasma concentration vs time profiles (Fig. 4). The time required to reach maximum plasma concentration was significantly shorter for the calcium salt. The calcium salt in the rat model appeared to be rapidly absorbed with sustained peak plasma levels. A separate bioavailability study had confirmed that the plasma concentration vs time profiles were similar for calcium and sodium salts. Intrinsic dissolution evaluated in a pH 7.2 Tris buffer with calcium and benzathine salts favored the calcium salt. The intrinsic dissolution for the calcium salt was 7.3 ± 1.6 mg/cm^2 per min. The intrinsic dissolution for the benzathine salt could not be determined under these conditions because its solubility was so low that no detectable drug concentrations were observed in samples from the dissolution medium.

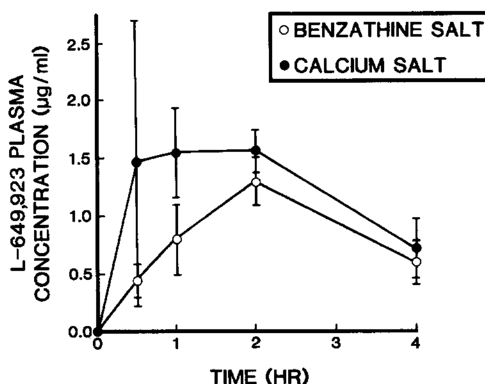

Fig. 4. Plasma concentration vs time profiles obtained for the calcium and benzathine salts of L-649,923 administered to the rat ($n = 4$) and dosed as an oral 1% Methocel suspensions at 20 mg/kg.

Although calcium salts have been employed in many marketed products, the benzathine salt has been used infrequently. One example in pharmaceutical preparations is benzathine penicillin (Schwartz et al., 1962). Because of its aqueous insolubility the benzathine salt has been used to stabilize penicillin in oral suspensions and reduce its absorption with intramuscular injectables. With L-649,923, there was concern regarding the benzathine salt's potential for slower absorption, with the associated implications of reduced onset of action and lower peak plasma concentrations. As a consequence, the calcium salt was chosen as the form of the drug for development.

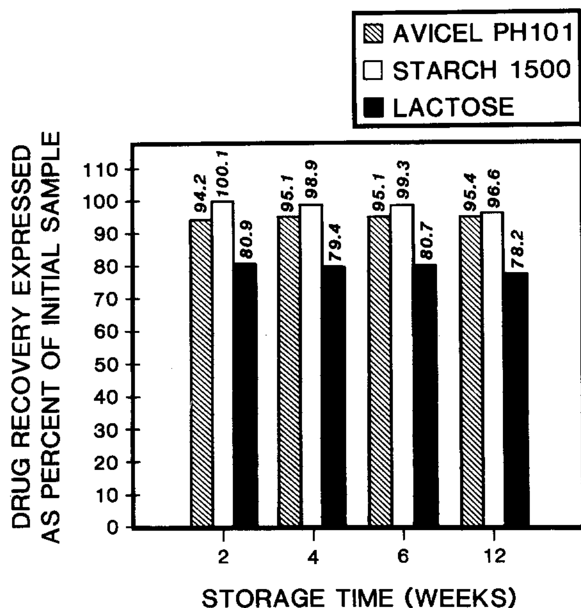

Fig. 5. Solid-state excipient compatibility studies under accelerated thermal stability conditions of 50°C. The percent of intact drug remaining in drug-excipient samples, corrected for the amount of drug loss in control samples stored under the same condition, is shown as a histogram. Samples were prepared with Avicel PH101, Starch 1500 and spray-dried lactose.

3.7. *Drug-excipient compatibility*

Drugs when in intimate contact with pharmaceutical excipients can either degrade or become physically associated with excipient. Often this results in difficulties with quantitative drug extraction (Cotton et al., 1987). In this case, potential drug-excipient interactions were assessed by determining the thermal stability of the calcium salt at 50 and 80°C in combination with several common pharmaceutical excipients used in solid dosage forms. Neat solid drug was the control. Good recovery was obtained when compared with the control samples under both temperature conditions with four of the five excipients studied. The single exception was with spray-dried lactose. Results obtained at 50°C for microcrystalline cellulose (Avicel PH101), pregelatinized starch and spray-dried lactose are shown in Fig. 5. Recovery from the lactose excipient was reduced by 20%. Because recovery was independent of time and no γ-lactone or other chromatographic peaks attributable to degradation products were observed in the HPLC chromatograms, the nonquantitative recovery in the presence of lactose was unlikely to be the result of degradation. Although there was no satisfactory explanation for the observed incomplete recovery of drug in the presence of lactose, it was not used for formulation development.

To monitor degradation, the γ-lactone concentrations in drug-excipient mixtures containing either pregelatinized starch or microcrystalline cellulose were compared with those obtained in the neat solid drug. Results obtained at 50°C did not indicate a significant acceleration of γ-lactone formation with time in the presence of either pregelatinized starch or microcrystalline cellulose.

As an extension of the drug-excipient interaction studies, aqueous granulations of drug and pregelatinized starch were prepared. Dry powder blends containing no lubricant were also prepared with drug and microcrystalline cellulose. These formulated materials were filled into hard gelatin capsules. Stability data obtained with three dose strengths at 40°C are listed in Table 4 (aqueous granulated material) and Table 5 (dry powder blends). Degradation was significantly greater with the aqueous granulation especially at a dose strength of 10 mg. Results indicated significant dose strength related degradation and, therefore, degradation dependent upon the drug-excipient ratio. These data suggest that moisture intro-

Table 4
Drug degradation at room temperature and at 40°C measured as γ-lactone formed

Storage condition	γ-Lactone formed (area percent) (Dose strength)		
	10 mg	50 mg	250 mg
RT/4 weeks	0.6	0.3	0.2
RT/12 weeks	2.0	0.6	0.4
40°C/4 weeks	5.5	3.0	2.4
40°C/12 weeks	9.0	4.4	3.3

Results are reported for three dose strengths each prepared as an aqueous granulation with drug and pregelatinized starch formulated as hard gelatin capsules. The γ-lactone formed is expressed as an HPLC area percent. RT, room temperature.

Table 5
Drug degradation at room temperature and at 40°C measured as γ-lactone formed

Storage condition	γ-Lactone formed (area percent) (Dose strength)		
	10 mg	50 mg	250 mg
RT/4 weeks	0	0.2	0.1
RT/12 weeks	1.0	0.6	0.3
RT/32 weeks	2.0	1.3	0.8
40°C/4 weeks	4.0	3.0	1.8
40°C/12 weeks	6.0	4.0	2.6

Results are reported for three dose strengths each prepared as dry powder blends of drug and microcrystalline cellulose formulated as hard gelatin capsules. The γ-lactone formed is expressed as an HPLC area percent. RT, room temperature.

duced by the excipient or during the granulation process adversely affected drug stability.

3.8. Trial capsule formulations

In an effort to minimize γ-lactone formation which may originate from the presence of free acid in capsule formulations, a specification of less than 1% free acid was recommended. Batches of the drug substance which met this specification were manufactured. These materials were used to prepare several trial formulations in hard gelatin capsules.

Incorporation of an alkalizing agent into the dry powder blend was investigated as a means of stabilizing the drug in the formulation. Powder blends containing microcrystalline cellulose (Avicel PH101) and 0.5, 1.0 and 1.5 drug/sodium carbonate molar ratios were prepared at a 50 mg dose strength. Similar dry powder blends were prepared with glycine and sodium phosphate dibasic as the alkalizing agents at molar ratios of 1.0. To test the blend for aqueous alkalinity, the pH of a saturated supernatant solution was determined in each case (Table 6). Results indicated that the saturated solutions obtained from the sodium carbonate powder blends were sufficiently basic at all the molar concentrations investigated. However, for the weaker bases, glycine and sodium phosphate, the pH was lower in the prepared saturated solutions and observed drug degradation slightly greater in the accelerated stability trials. Results obtained from samples at room temperature were independent of the amount of added alkalizing agent.

Further work examined the effect of water content on drug degradation. A 50 mg formulation was prepared from an aqueous pregelatinized starch granulation. Drug degradation was not significant during preparation of the granulation but subsequent stability was inferior to other formulation alternatives (Table 6). To minimize moisture content, dry powder blends were prepared with Avicel PH103 (2.5–3.0% moisture as compared with Avicel PH101 at 5.0%). The least drug degradation was observed with the formulation containing low moisture Avicel PH103 and

Table 6
Drug degradation measured as γ-lactone formed

Alkalizing agent	pH of a supernatant saturated solution	γ-Lactone formed (area percent)	
		40°C/12 weeks	RT/30 weeks
Aqueous starch granulation – no alkalizing agent	not measured	5.0	not determined
No alkalizing agent with Avicel PH 101 (dry powder blend)	6.9	4.4	not determined
0.5 molar equivalent sodium carbonate/Avicel PH 101	9.5	2.5	0.6
1.0 molar equivalent sodium carbonate/Avicel PH 101	9.7	2.4	0.6
1.5 molar equivalent sodium carbonate/Avicel PH 101	9.9	2.4	0.6
1.0 molar equivalent glycine/Avicel PH 101	7.3	2.8	0.8
1.0 molar equivalent sodium phosphate dibasic/Avicel PH 101	7.8	3.0	0.6
1.0 molar equivalent sodium carbonate/Avicel PH 103	not measured	1.2	0.2

Hard gelatin capsule formulations contained dry powder blends with different alkalizing agents present. γ-Lactone formed is expressed as an HPLC area percent. In all cases, the dose strength of formulations described above is 50 mg. The use of low moisture content microcrystalline cellulose (Avicel PH103) improved stability. RT, room temperature.

Table 7
Drug degradation observed for two 50 mg hard gelatin capsule formulations stored at room temperature

Storage time	γ-Lactone content (area percent)	
	Avicel PH101 powder blend	Avicel PH103, sodium carbonate blend
Initial	0.8	0
1 month	1.0	0
3 months	1.2	0.4
6 months	1.6	0.2

The formulation containing the alkalizing agent is significantly more stable than the formulation containing only a dry powder blend of drug and microcrystalline cellulose. The γ-lactone content, expressed as an HPLC area percent, was used as a measure of drug degradation.

sodium carbonate as the alkalizing agent. A comparison of the γ-lactone content after 6 months at room temperature with two capsule formulations, one containing a dry powder blend with Avicel PH101 and a second containing a dry powder blend with Avicel PH103 and sodium carbonate, indicated significant improvement in stability for the latter formulation (Table 7).

3.9. Trial tablet formulations

The moisture content of hard gelatin capsules is 12-15%. This water can be extracted by hygroscopic solids. It will migrate into the solid capsule contents. Capsule brittleness was observed for hard gelatin capsule formulations of L-649,923, especially at high dose strengths, a phenomenon attributable to the loss of the plasticizing properties of water. This suggested that a more stable dosage form was attainable if the drug was formulated as a direct compression tablet. Small trial formulations were prepared using a single punch tablet press at dose strengths of 250 and 375 mg. In each case the drug was dry blended with equimolar sodium carbonate and Avicel PH103. These formulations had a hardness of 7-8 kp with good disintegration and dissolution properties. Moisture in the initial compressed tablets was determined using a standard procedure (USP XXII, 1990). The losses on drying for 250 and 375 mg formulations were 2.2 and 2.6%, respectively. They were stored in amber glass bottles with and without desiccant under conditions of accelerated thermal stability. The observed degradation was minor and not significantly different with or without desiccant in the storage container. Results from these studies (Table 8) established that drug degradation could be minimized with a calcium salt direct compression tablet when the issues of free acid, excipient moisture content and alkalinity were addressed.

3.10. The mechanism of degradation

The two most common routes of degradation in pharmaceutical preparations are either hydrolytic or oxidative mechanisms. Oxidative mechanisms generally result in many degradation products at low concentrations (Cotton and Down, 1988). Material accountability is difficult to obtain and the degradative process must be followed by the loss of intact drug. As with the case of L-649,923, hydrolytic mechanisms often result in one or two degradation products. Good material accountability is often is achieved and, in such cases, the degradative process can be monitored more precisely by following the increase in concentration of the products of degradation.

Understanding the degradation mechanism can help in designing the formulation to minimize drug loss. For L-649,923 the internal esterifica-

Table 8
Accelerated thermal stability data for direct compression tablet formulations of L-649,923 calcium salt optimized to minimize drug degradation

Storage conditions	γ-Lactone content (area percent)	
	250 mg dose	375 mg dose
Initial	0.1	0.1
30°C/1 month	0.1	0.2
40°C/1 month	0.1	0.2
RT/3 months	0.2	0.3
30°C/3 months	0.2	0.3
40°C/3 months	0.2	0.3
RT/6 months	0.2	0.2
30°C/6 months	0.1	0.2
40°C/6 months	0.2	0.2

RT, room temperature.

tion process forming the γ-lactone as the principal product resembles an acid-catalyzed esterification mechanism occurring in aqueous solution. The solution chemistry of esterification and hydrolysis has been thoroughly investigated. Lactonization in aqueous solution is well defined (Gould, 1960). To confirm the solution chemistry with L-649,923, a 50 mg/ml sodium salt solution was prepared in a sodium acetate buffer at pH 7.8 and stored at room temperature, 40 and 60°C for 1 month. The equilibrium γ-lactone concentrations determined by HPLC were 7.3, 11.9 and 14.8% of the initial drug concentration, respectively. Further evidence of the importance of moisture in the drug degradation process was obtained by adding water to previously dried and weighed samples of L-649,923, storing the samples at 40°C for 3 months and determining γ-lactone content by HPLC. When 8.4, 4.3, 2.8 and 1.0% w/w moisture were added, the amount of γ-lactone formed was 1.4, 0.6, 0.03 and 0.08%, respectively. Moisture content above 3% significantly affected drug degradation. A proposed simplified mechanism of drug degradation to the γ-lactone in sorbed moisture is shown schematically in Fig. 6. Previously, the hydrolytic degradation of aspirin to salicylic acid had been explained by a similar solution process in sorbed moisture at the surface of the drug substance (Leeson and Mattocks, 1958).

The L-649,923 calcium salt is sufficiently hygroscopic. It is possible for it to sorb water, especially at high relative humidity, to account for significant surface moisture. Moisture may penetrate the solid-state structure and plasticize the amorphous material (Ahlneck and Zografi, 1990). Evidence from analysis of water sorption isotherms has suggested that water sorbed to microcrystalline cellulose may exist in three different associated states: tightly bound, somewhat loosely bound and bulk water (Zografi et al., 1984). Moisture associated with formulated materials containing microcrystalline cellulose may also interact with the surface of the drug substance resulting in either plasticization or dissolution. The amorphous calcium salt has sufficient solubility to form a solution with a significant drug concentration in associated moisture. In addition, plasticization of surface layers of drug particles may increase molecular mobility and cause the material to behave in a manner somewhat analogous to that of a supersaturated solution. Such surface dissolution and plasticization behavior has been documented and discussed elsewhere (Car-

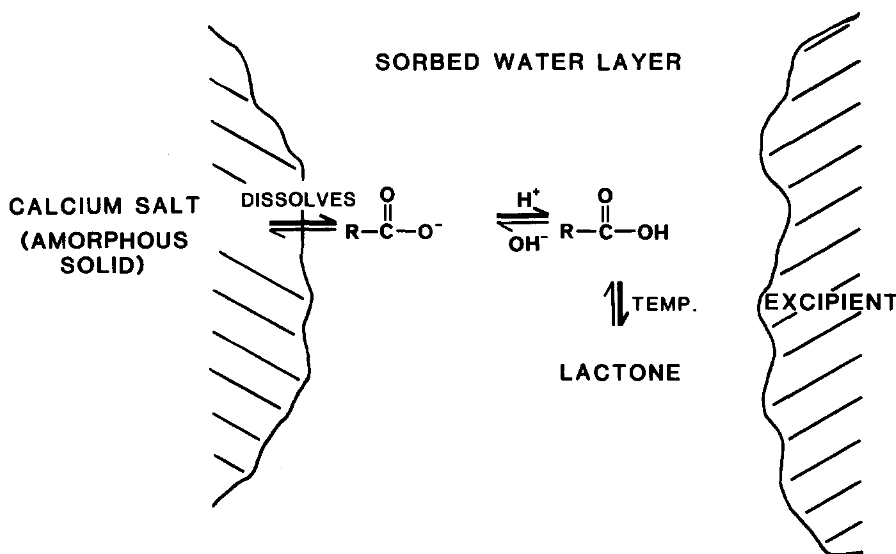

Fig. 6. A schematic of the proposed moisture-induced degradation of the amorphous calcium salt of L-649,923 to its *cis* γ-lactone.

stensen et al., 1969; Kontny et al., 1987; Ahlneck and Zografi, 1990). Residual free acid may also contribute to the acidity in the moisture layers. The resulting weakly acidic condition will promote γ-lactone formation. Rather than a solid-state degradation mechanism, the evidence suggests an acid-catalyzed solution esterification occurring in the microenvironment of the plasticized solid drug substance where molecular mobility may resemble that of a concentrated solution.

This solution degradation mechanism is consistent with the observation that γ-lactone formation approaches an apparent temperature-dependent equilibrium condition after several weeks. It predicts that the calcium salt can be stabilized and γ-lactone formation minimized in the formulation if (i) the amount of free acid in the drug substance is minimized, (ii) an alkalizing agent is added to the solid material to ensure that the pH of the microenvironment is basic, (iii) an aqueous granulating process is avoided and (iv) excipients having a low water content are used. This postulated mechanism and its predictive responses formed the basis for developing a chemically stable solid oral dosage form. In the process of stabilizing the formulation, the general utility of considering the mechanisms of degradation during formulation development has been demonstrated. Evaluating the causes of degradation can help in defining those conditions which maximize the shelf life of the product.

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