Maillard Reaction of Lactose and Fluoxetine Hydrochloride, a Secondary Amine

DAVID D. WIRTH*, STEVEN W. BAERTSCHI, ROSS A. JOHNSON, STEVEN R. MAPLE, MARYBETH S. MILLER, DIANA K. HALLENBECK, AND STEPHEN M. GREGG

Contribution from the *Lilly Research Laboratories, Eli Lilly and Company, Lafayette, Indiana 47905*

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Abstract □ Analysis of commercially available generic formulations of fluoxetine HCl revealed the presence of lactose as the most common excipient. We show that such formulations are inherently less stable than formulations with starch as the diluent due to the Maillard reaction between the drug, a secondary amine hydrochloride, and lactose. The Amadori rearrangement product was isolated and characterized; the characterization was aided by reduction with sodium borohydride and subsequent characterization of this reduced adduct. The lactose–fluoxetine HCl reaction was examined in aqueous ethanol and in the solid state, in which factors such as water content, lubricant concentration, and temperature were found to influence the degradation. *N*-Formylfluoxetine was identified as a major product of this Maillard reaction and it is proposed that *N*-formyl compounds be used as markers for this drug–excipient interaction since they are easy to prepare synthetically. Many characteristic volatile products of the Maillard reaction have been identified by GC/MS, including furaldehyde, maltol, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one. Close similarity between the degradation products of simple mixtures and formulated generic products was found; however, at least one product decomposed at a rate nearly 10 times that predicted from the simple models. Maillard products have also been identified in unstressed capsules. The main conclusion is that drugs which are secondary amines (not just primary amines as sometimes reported) undergo the Maillard reaction with lactose under pharmaceutically relevant conditions. This finding should be considered during the selection of excipients and stability protocols for drugs which are secondary amines or their salts, just as it currently is for primary amines.

Introduction

The Maillard reaction is named after Louis Maillard, who reported over 80 years ago that some amines and reducing carbohydrates react to produce brown pigments.[1] It has been extensively studied and reviewed, especially in the food and nutrition literature.[2] More recent reviews cover not only the chemistry of foods but human physiology as well.[3] The first product of this reaction is a simple glycosylamine,[4] which readily undergoes the Amadori rearrangement to produce 1-amino-1-deoxy-2-ketoses.[5] The large body of literature on these reactions is due to the multitude of possible reaction pathways and products, including fragmentations of the carbohydrates and formation of aromatic compounds from cyclization/dehydration processes.[6] Reducing disaccharides also undergo this reaction,[7] and it is a well-documented process for the degradation of lactose during the heating of milk.[8] Reducing carbohydrates such as glucose, maltose, and lactose are substrates for the Maillard reaction since their cyclic tautomers are in equilibrium with their more reactive aldehyde forms; nonreducing carbohydrates such as mannitol, sucrose, and trehalose are not subject to Maillard reactions. Although early scientists believed that only primary aromatic amines were capable of this reaction, subsequent research has shown that nearly all primary and secondary amines, aromatic or aliphatic, are capable of this reaction.[9] Amino acids and proteins are also substrates for the Maillard reaction.[10] The impact of these reactions on the stability of pharmaceuticals has also been known for some time and was recently reviewed.[11]

Prozac, the world's largest selling antidepressant, is a selective serotonin reuptake inhibitor;[12] it has been used by over 25 million people in more than 90 countries. Its active ingredient is fluoxetine hydrochloride, **1**, and the diluent is starch. Within the past few years, many generic formulations containing fluoxetine HCl have been manufactured and sold in countries lacking patent protection or in which the patents have expired. We have recently examined several dozen of these products and find that a majority of them contain lactose as their primary diluent. Lactose is widely used as a diluent for capsules and tablets due to its low price, high purity, and excellent compression and stability characteristics. Although its ability to undergo nonenzymatic browning (Maillard) reactions has long been known to formulation scientists, some reports have suggested this interaction to be largely restricted to primary amines.[13] The origin of this view is likely founded in early literature reports of the slower browning of glucose mixtures of *N*-methyl glycine as compared to glycine itself[14] and work on the browning of mixtures of lactose and methylated amphetamines,[15] studies which measured optical density only, not characteristic Maillard products. Although these reports showed faster rates of browning of mixtures of reducing carbohydrates with primary amines as compared to secondary ones, they do not support the conclusion that secondary amines should generally be incapable of the Maillard reaction.

The generally accepted mechanism of glycosylation and the Amadori rearrangement are summarized in Scheme 1 for a secondary amine.[16] The imminium ion intermediate can be formed from either primary or secondary amines (but not tertiary ones) and is the key intermediate in both closure to the glycosylamine and deprotonation to the enol version of the Amadori rearrangement product, ARP. The multitude of individual steps in this mechanism allows for considerable variation in the kinetics. For example, the hydrolysis of the glycosylamine of benzylamine and glucose is faster at pH 5 than hydrolysis of the corresponding glycosylamine with piperidine, but the reverse order of reactivity is observed at pH 9.[17] Kinetic studies of the Maillard reaction have been reviewed and will not be explored in depth in this paper.[18] In view of the well-

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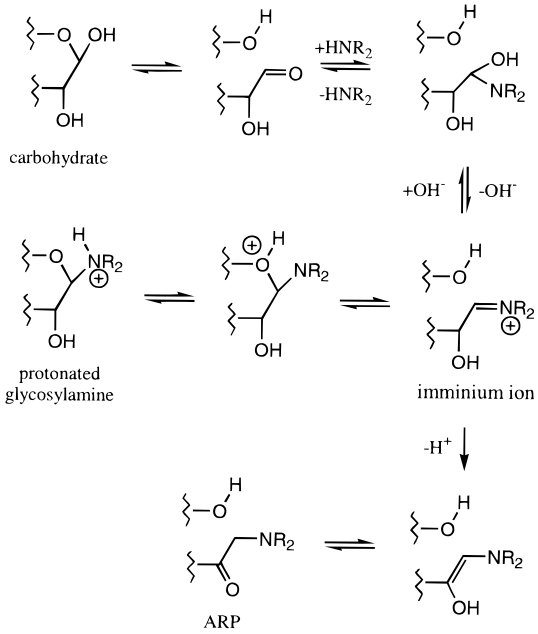


Scheme 1—Mechanism of glycosylation and Amadori rearrangement with secondary amines.

known interaction of reducing carbohydrates and secondary amines, as reported in the carbohydrate and food science literature,[19] we have investigated the Maillard/Amadori reactions of the lactose–fluoxetine system and report our findings here. Detailed mechanistic work such as identification of the rate-determining step and full kinetic analysis as a function of the amine, carbohydrate, and reaction conditions are left for future studies.

Experimental Section

Materials—Fluoxetine HCl and Prozac were obtained from Eli Lilly and Co. generics A, N, O, and Z were obtained in pharmacies outside the United States and were unexpired formulated 20 mg products. Lactose monohydrate was obtained from Aldrich Chemical Co, Milwaukee, WI, and spray-dried lactose was from Foremost Ingredients Group, Baraboo, WI. The anhydrous lactose forms were obtained from Sheffield Products, Norwich, NY. The acetonitrile was spectroscopic grade, obtained from EM Science, Gibbstown, NJ. Reagent grade trifluoroacetic acid was obtained from Aldrich Chemical Co. and was distilled prior to use. Other chemicals and the authentic samples of compounds listed in Table 6 were purchased from Aldrich Chemical Co. and were used as received. Ethanol was anhydrous and denatured with 5% methanol.

NMR—Nuclear magnetic resonance (NMR) spectroscopy for compounds **1**, **2**, **3**, and **6** was carried out on a Bruker AC300 spectrometer with ^{1}H and ^{13}C operating frequencies of 300.13 and 75.46 MHz, respectively. Additional experiments on compound **6** were carried out on a Bruker AMX500 with ^{1}H and ^{13}C operating frequencies of 500.14 and 125.77 MHz, respectively.

GC/MS—Gas chromatography/mass spectrometry was performed on a MicroMass TRIO II quadrapole mass spectrometer controlled using the Masslynx operating system. The instrument was equipped with a Hewlett-Packard Model 5890 gas chromatograph which was fitted with a Scientific Instruments Services short path thermal desorption Model TD 4 system and CryoTrap unit. The mass spectrometer was operated in the EI+ mode and calibrated daily against FC-43. Mass spectral data were obtained in the centroid mode by scanning the mass range from 33 to 600 amu at a rate of 1260 amu/s and using an interscan time delay of 0.05 s.

For the analysis of products from solid-state samples, the contents of one 20 mg capsule of fluoxetine HCl product or mixtures of 20 mg of bulk fluoxetine HCl and 200 mg of lactose were added to a 10 mL glass vial which was crimp sealed with a Teflon-lined rubber septum. The vials were heated at 100 °C for 20 h. A stainless steel GLT short path thermal desorption tube packed with 100 mg of Tenax-TA which had been preconditioned with helium purge at 250 °C and fitted with a needle was inserted into the vial. A second needle connected to a helium source was inserted into the vial and the flow rate set at approximately 7 mL/min. The vial headspace gases were purged onto the Tennax-TA tube for either 5 or 60 min. The GLT tube was then transferred to the short path thermal desorption unit for desorption onto the gas chromatographic column. The following conditions were used for the transfer and chromatographic analysis. Sample transfer: gas purge time, 0.5 min; desorption time, 5 min; GLT desorption temperature, 100 °C initial then 40 °C/min to 200 °C; column cryotrap at −100 °C. GC conditions: injector, 150 °C; transfer line to mass spectrometer, 300 °C; split flow, 7 mL/min or 50 mL/min; the cryotrap was heated rapidly to 250 °C to desorb material onto the GC column; oven temperature program, 40 °C for 3 min then 10 °C/min to 300 °C and hold 3 min; chromatographic column, DB-1, 30 m × 0.25 mm i.d. and 1.0 mm film.

HPLC—High performance liquid chromatography was performed with a Spectra Physics SP8700XR pump, a Spectroflow 757 detector (Anspec) set at 260 nm, a ChromJet integrator by Spectra Physics, and an Alcott 728 autosampler incorporating a Valco injection valve with a 10 μL fixed loop. The column was a Zorbax RX-C8, 25 cm by 4.6 mm, 5 μm particles (Mac-Mod Analytical, Inc., Chadds Ford, PA) maintained at ambient temperature. The flow rate was 1.00 mL min^{-1} throughout. The two eluting solutions used were A = 0.07% trifluoroacetic acid (TFA) in water, v/v; B = 0.07% TFA in acetonitrile. The elution program started at 80:20 A:B for 5 min, ramped linearly to 15:85 A:B at 30 min, held there until 35 min, and returned to 80:20 A:B at 40 min. Injections were made no sooner than every 50 min. The approximate elution times are **1** at 21 min, **2** at 18.4 min, **3** at 27 min, and **4** at 17.9 min. For MS detection, samples were analyzed on MicroMass (formerly Fisons) Quattro II and Platform II LC/MS systems in the positive ion electrospray mode, scanning from 100 to 1000 amu, generating centroid data. Further details and example chromatograms have been published elsewhere.[20] This method allows for the detection and quantitation of impurities which span a wide range of polarities, including nonpolar compounds which are not eluted using the isocratic US Pharmacopeia method.[21]

Lactose–Fluoxetine Amadori Rearrangement Product (ARP, 2)—In a 500 mL round-bottom flask equipped with an overhead stirrer, thermometer, and condenser were combined 20 g of α-D-lactose monohydrate (55.5 mmol), 10 g of fluoxetine HCl (28.9 mmol), 250 mL of ethanol, 120 mL of dimethylacetamide, and 4 mL of triethylamine (28.7 mmol). The nearly homogeneous mixture was stirred at reflux for 24 h. The reaction mixture was filtered at ambient temperature and the wetcake was washed with 50 mL of ethanol. The filtrate was evaporated in vacuo to a thick brown solution (112 g), to which were added 300 mL of ethyl acetate, 300 mL of 20% aqueous sodium chloride, and 30 mL of ether. The pH was adjusted to 11 with 50% sodium hydroxide. White solids which formed during the pH adjustment were filtered and the layers were separated. The bottom layer was discarded and the upper layer was set aside. To the milky middle layer were added 300 mL of ethyl acetate and 30 mL of ether. The layers were separated and the combined organic layers were evaporated in vacuo to a thick brown oil (42.8 g), to which were added 175 mL of 20% aqueous sodium chloride, 400 mL water, and 550 mL chloroform. The pH was adjusted to 1.5 with concentrated HCl. The layers were separated and to the aqueous layer were added 60 g of NaCl, 500 mL of ethyl acetate, and 50 mL of ether. The pH was adjusted to 10.6 with 50% NaOH, and the layers were separated. The ethyl acetate layer was evaporated in vacuo to a residue (4.7 g) which was 85% pure by HPLC analysis at 260 nm. Standard ^{1}H and ^{13}C NMR spectra were acquired on a sample of **2** prepared by dissolving 50–60 mg of the material in DMSO-d_6. A partial list of assignments has been made based on comparison to fluoxetine and is shown in Table 1. Flow-injection mass spectrometry indicated the molecular weight to be 633 ($M + H^+$, m/z 634). Accurate mass FAB-MS measurements of the protonated molecular ion ($M + H^+$) indicated the elemental composition to be $C_{29}H_{39}NO_{11}F_3$ (calcd 634.2475, measured 634.2487).

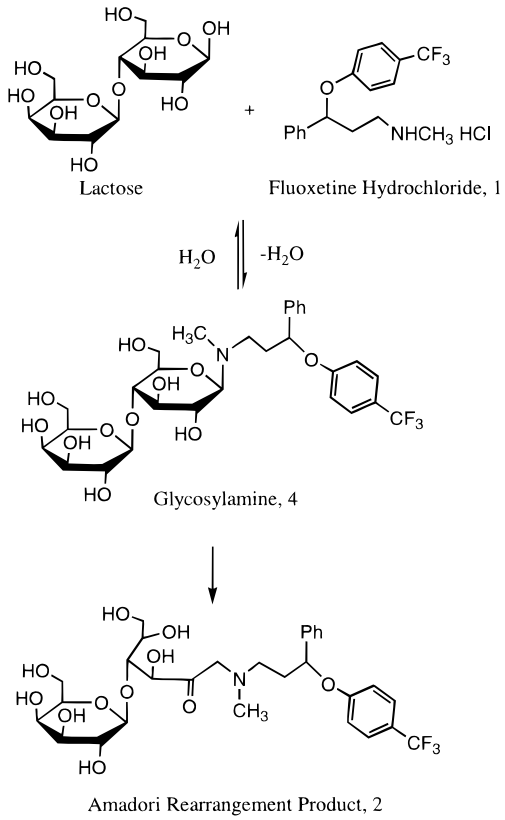
Reduced Adduct (6)—In a 50 mL round-bottom flask equipped with an overhead stirrer, a thermometer, and a condenser were combined 1.5 g of ARP, **2** (2.37 mmol), 15 mL of ethanol, and 1.79

g of $NaBH_4$ (47.4 mmol, 20 equiv). The mixture was stirred at reflux for 1 h and at 70 °C for 1 h. The mixture was cooled to ambient temperature and 10 mL of ethanol was added. Excess $NaBH_4$ was quenched by the dropwise addition of 5 mL of acetone at 5 °C. The mixture was evaporated to a wet residue under vacuum at 35 °C. To the residue were added 200 mL of 20% aqueous sodium chloride and 200 mL of $CHCl_3$, and the pH was adjusted to 2.2 with concentrated HCl. The layers were separated (after settling overnight). To the $CHCl_3$ layer were added 100 mL of $CHCl_3$ and 200 mL of water, and the pH was adjusted to 2.2 with concentrated HCl. The layers were separated, to the water layer were added 300 mL of ethyl acetate and 30 mL of ether, and the pH was adjusted to 10.6 with 50% NaOH. The layers were separated, and the ethyl acetate layer was concentrated under vacuum, producing 0.4 g of residue which contained 80% of the desired reduced fluoxetine–lactose adduct, **6**, by HPLC analysis at 260 nm. Extensive NMR experiments to confirm the structure of **6** were carried out on a sample prepared by dissolving ~60 mg in DMSO-d_6. The experiments performed were ^{13}C DEPT (distortionless enhancement by polarization transfer), 2D $^1H-^1H$ COSY (correlation spectroscopy),[22] 2D $^1H-^{13}C$ HMQC (heteronuclear multiple quantum coherence),[23] and 2D $^1H-^{13}C$ HMBC (heteronuclear multiple bond correlation).[24] The NMR assignments are shown in Table 1. Mass spectrometry (infusion, electrospray) verified the molecular weight by the presence of a large M + 1 ion at 636 amu.

***N*-Formylfluoxetine (3)**—Acetic formic anhydride was prepared by stirring acetic anhydride (4.87 mL, 51.6 mmol) and formic acid (7.78 mL, 206 mmol) in a beaker for 5 min. Fluoxetine HCl (4.5 g, 12.9 mmol) and methylene chloride (30 mL) were placed in a 100 mL round-bottomed flask. Sodium hydroxide (1 M, 30 mL, 30 mmol) was added. The solution was stirred at room temperature for 5–10 min. The layers were separated, and the organic layer was dried with sodium sulfate and filtered. The filtrate was placed in a 100 mL round-bottomed flask, and the acetic formic anhydride was added. The reaction was stirred at room temperature for 5 h and extracted with a 10% sodium bicarbonate solution (3 × 50 mL). The organic layer was dried with sodium sulfate and filtered. The filtrate was concentrated to an oil (1.5 g) which solidified upon standing (mp 65–67 °C). The purity by the gradient HPLC method was 98.9%. The major and minor rotomers about the amide bond are identified in the following NMR spectra. ^1H NMR (300 MHz, CD_2Cl_2) 8.03–7.96 (dd, 1 H), 7.52–7.43 (d, 2H), 7.41–7.23 (m, 5H), 6.98–6.88 (dd, 2H), 5.30–5.22 (m, 1H, minor), 5.22–5.16 (m, 1H, major), 3.62–3.48 (m, 2H, major), 3.44–3.16 (m, 2H, minor), 2.94 (s, 3H, minor), 2.88 (s, 3H, major), 2.32–1.96 (m, 2H); ^{13}C NMR (CD_2Cl_2, major rotomer) 162.79, 160.56, 140.55, 129.28, 128.51,127.15, 126.14, 122.99, 116.16, 77.44, 46.22, 37.23, 29.56; (minor rotomer) 162.75, 160.84, 141.01, 129.15, 128.34, 127.05, 126.24, 123.42, 116.25, 78.51, 41.52, 36.11, 34.89; ^{19}F NMR (282 MHz, CD_2Cl_2) −62.14, −62.2; IR (KBr) 2934, 2875, 1689, 1334, 1249, 1117, 847 cm^{-1}; MS (CI^+) *m/e* 338 (25), 176 (100), 143 (43).

Reactions in Aqueous Ethanol—Fluoxetine HCl (0.50 g, 1.45 mmol) and lactose monohydrate (5.0 g, 13.9 mmol) were combined with 5.0 mL of ethanol and 7.5 mL of water in each of four separate flasks. The mixtures were heated to 60 °C and vigorously stirred with a mechanical agitator. Potassium hydroxide (85% assay, amounts of 0, 7, 47, or 100 mg) was added, and the reactions were monitored by the above HPLC method. The equivalents of KOH based on the fluoxetine and the results are given in Table 2. The apparent pH (called pH*) was measured by direct insertion of a calibrated glass electrode into the warm reaction mixture. Samples were diluted 1:5 with eluent for HPLC measurement. The fluoxetine concentration was measured versus an external standard and the mole percent concentrations of **2** and **3** were taken as equal to their area percent values since their molar response factors were measured on authentic material and found to be within 5% of that of fluoxetine itself.

Solid-State Screening Studies—For the screening studies, 5.0 g of lactose and 0.50 g of fluoxetine HCl were combined and mixed one of three ways, described here according to their designation in Table 3. Those tumbled were placed in capped glass bottles and strapped to a horizontal rotating rod for 40 min. Those that were ground were mixed thoroughly in a standard glass mortar and pestle for several minutes. To those labeled "wet grind" was added 0.5 g water before the mortar and pestle grinding operation. Some experiments, identified with a Y in the column



Scheme 2—Maillard reaction of lactose and fluoxetine HCl.

headed Mg stearate, were repeated with 0.04 g of magnesium stearate added before the blending or grinding operation. Four commonly available types of lactose were screened: crystalline monohydrate; a mixture of crystalline and spray-dried monohydrate; and two particle size grades of anhydrous lactose, granular and finely milled. After mixing, the samples were heated in an oven at 98 °C for 24 h in glass vials with loose-fitting plastic caps. The samples were analyzed with the gradient HPLC method and the results are reported using fluoxetine as the standard. Since compounds **2** and **3** have the same molar response as fluoxetine, the levels given for these impurities are molar yields. The total impurity level is estimated by assuming that the molar responses of all impurities are equal to fluoxetine's and thus can be compared on a relative basis only.

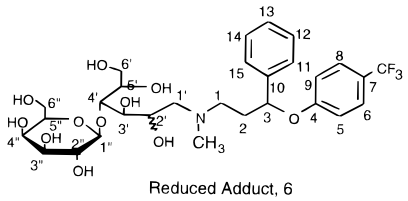
Solid-State Kinetic Studies—Lactose (5.0 g, in separate experiments, the monohydrate, anhydrous lactose, and monohydrate containing an additional 0.10 g of magnesium stearate) and fluoxetine HCl (0.50 g) were mixed thoroughly with a mortar and pestle and 1.8 g of the mixtures was added to each of three 10 mL glass vials. The vials were lightly capped and placed in equilibrated ovens at 75, 85, or 95 °C. Samples of the solids were removed at 23, 47, 95, 175, and 287 h for the 75 °C experiment; at 6, 23, 47, 99, and 122 h for the 85 °C experiment; and at 2, 6, 13, 21, and 28 h for the 95 °C experiment. The samples were assayed as described for the screening studies.

Results and Discussion

Preparation and Characterization of Amadori Rearrangement Product (ARP), 2—The Maillard reaction between fluoxetine and lactose to produce the ARP, **2**, is shown in Scheme 2. An authentic sample of 2 was obtained by reaction of lactose and fluoxetine HCl in *N*,*N*-dimethylacetamide and ethanol as solvent with triethylamine as the base. It was purified by extractions but was not crystallized.

Although the structure of **2** is shown as acyclic, it exists in solution as a mixture of pyranose and furanose forms both of which can be diastereomeric.[25] For this reason,

Table 1—NMR Data for Compounds 1, 2, and 6



Reduced Adduct, 6

	fluoxetine, **1**		ARP, **2**		reduced adduct, **6**[a]	
position[b]	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	44.99	2.99			53.88	3.29
					51.30	3.26
2	34.01	2.31, 2.23		2.35, 2.28	31.88	2.36, 2.19
3	76.38	5.74		5.58	77.00	5.53
4	160.09	—	160.66	—	160.10	—
5, 9	116.28	7.08	116.20	7.06	116.36	7.07
6, 8	126.77	7.55	126.82	7.55	126.93	7.56
7	121.36	—	120.98	—	121.56	—
10	139.97	—	141.21	—	139.87	—
11, 15	125.94	7.42	126.19	7.46	126.07	7.41
12, 14	128.74	7.36	128.58	7.36	128.88	7.37
13	128.01	7.27	127.64	7.28	128.22	7.29
N–CH_3	32.25	2.51			41.45	2.84
					39.21	2.78
CF_3	124.38	—	124.53	—	124.47	—
1′					58.95	3.44, 3.04
					58.06	3.33, 3.15
2′					64.48	4.16
3′					71.41	3.58
					71.08	3.52
4′					75.32	3.39
5′					68.34	3.58
6′					61.06	3.54
1″				4.28	103.97	4.24
2″					70.65	3.33
3″					73.44	3.30
4″					70.01	3.65
5″					77.56	3.75
6″					62.20	3.66

[a] Proton and carbon chemical shifts are taken from the HMQC data. [b] Numbering for **1** and **2** are similar to that shown for **6**.

complete characterization of **2** was not accomplished. Rather, sufficient mass spectral and NMR characterization was performed to be reasonably confident of the structure and this was supplemented with more complete characterization on the alcohol **6**, prepared by reduction of **2** with sodium borohydride. The NMR assignments can be found in Table 1. Several key elements were needed to confirm the structure of **6**. Specifically, the combination of proton−proton coupling (COSY) and proton−carbon long-range coupling (HMBC), along with basic proton and carbon chemical shift information, was used to show the following (refer to Table 1). First, the fluoxetine moiety in the molecule is intact. Second, the *N*-methyl protons of fluoxetine show coupling to the adjacent methylene C1 of fluoxetine, and more importantly to the methylene C1′ of the lactose moiety, thereby establishing the site of union between the fluoxetine and the lactose. Finally, the galactose unit of the lactose has been retained. The anomeric carbon at 1″ maintains the downfield chemical shift of 103.9 ppm seen in lactose.[26] The HMBC experiment also shows coupling from the proton at 3.39 ppm of 4′ to the 1″ carbon. Similarly, the 1″ proton at 4.24 ppm shows coupling to the 4′ carbon at 75.3 ppm.

The NMR data is supported by the mass spectral work, which identified the molecular weight of the sample to be 635 amu, consistent with the proposed structure of **6**. This is also exactly two mass units higher than the molecular weight for compound **2**, consistent with the reduction of the ketone with sodium borohydride.

Table 2—Apparent Rates of Product Formation for the Reaction of Fluoxetine HCl and Lactose in Aqueous Ethanol at 60 °C

		percent per hour	
pH*	equiv KOH	ARP, **2**	*N*-formyl-fluoxetine, **3**
5.0	0	0.0014	0
7.0	0.07	0.084	0
8.0	0.50	0.31	0.040
8.4	1.05	0.16	0.22

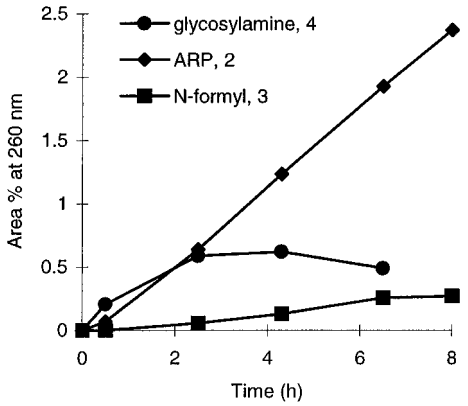


Figure 1—HPLC data for the reaction of fluoxetine HCl, lactose, and KOH at pH* 8.0, 60 °C in aqueous ethanol.

Reactions in Aqueous Ethanol—Fluoxetine HCl and lactose monohydrate were heated to 60 °C in aqueous ethanol and the concentrations of **2** and **3** were measured as a function of the amount of added base and time. Apparent rates for the appearance of the two products are given in Table 2. They were obtained from a linear fit to their concentrations obtained by HPLC using at least five data points but excluding the origin, since a slight induction period was evident in the reactions. The linear fits had coefficients of determination (R^2) of 0.97 or greater. Data for only the first few percent conversion were used, where the order is effectively zero with all reactants so that apparent rates in (mole) percent per hour are reported rather than rate constants to add relevance.

Without addition of KOH, the apparent pH (pH*) was 5.0 and the reaction was very slow. However, addition of only 0.07 equiv of KOH (pH* = 7.0) increased the rate of appearance of **2** by a factor of 60. At higher pH values, the rate of formation of **2** increased further and the production of *N*-formylfluoxetine, **3**, was observed. Representative data from pH* = 8.0 is shown in Figure 1. The peak assigned to the glycosylamine, **4**, eluted just before **2** and was assumed to be this compound based on the fact that it was the first visible product of the reaction, was quickly converted to **2**, and had a molecular weight of 633, as determined by LC/MS analysis. It is clear from this solution-phase study that the Maillard and Amadori reactions of fluoxetine HCl and lactose are viable processes and that they are readily catalyzed by base.

Formamides and acetamides are well-known products of the decomposition of Amadori rearrangement products, both for primary[27] and secondary amines.[28] Although both formylation and acetylation of fluoxetine have been observed in the studies reported here, the former has been observed throughout, often as the primary path of decomposition, whereas only small amounts of acetylation have been observed. Two paths to formamides in the Maillard

Table 3—Solid Phase Screening Experiments for Mixtures Heated at 98 °C for 24 h

lactose type	mixing mode	Mg stearate	% Impurities total	% Impurities ARP, **2**	% Impurities *N*-formyl-fluoxetine, **3**
monohydrate	tumble	N	0.48	0.073	
		Y	0.46	0.16	0.023
	grind	N	0.48	0.089	
		Y	0.6	0.21	0.039
	wet grind	N	6.13	3.23	0.02
		Y	17.3	8.56	0.4
spray-dried	tumble	N	1.2	0.26	
		Y	0.92	0.4	0.036
	grind	N	1.48	0.45	0.02
		Y	1.44	0.41	0.12
	wet grind	N	22.8	5.1	0.078
		Y	24.2	13	0.48
anhydr gran	tumble	N	0.46	0.072	
	grind	N	0.5	0.079	
	wet grind	N	3.11	1.51	0.014
anhydr milled	tumble	N	0.44	0.079	
	grind	N	0.53	0.107	
	wet grind	N	2.06	0.86	0.011

reaction are generally considered: the oxidative cleavage of the glycosylamine (or a smaller fragment of it)[3] or the acylation of free amine with a 1,2-dicarbonyl compound, especially glyoxal.[29] In the present case, addition of 0.1 equiv of glyoxal to a 0.1 M solution of fluoxetine HCl containing 0.5 equiv of KOH in aqueous ethanol at 60 °C did produce *N*-formylfluoxetine, **3**, as well as a few other unidentified products. This is consistent with but does not prove that glyoxal is the precursor to *N*-formylfluoxetine in the solution or solid-phase experiments. Similar spiking experiments with formic acid or glyoxalic acid produced no new products.

Solid State Reactions, Monitored by HPLC—Having shown that the Maillard reaction of lactose and fluoxetine occurs in solution and that **2** and **3** are the expected products, attention turned to the more pharmaceutically relevant case of solid-phase reactions. The simplest experiment to identify such a drug−excipient interaction is visual observation of a heated mixture.[30] Such an approach was used over 30 years ago to warn formulation scientists of the browning of lactose formulations of drugs which contain primary and secondary amine functionality.[31] Indeed, when lactose and fluoxetine HCl as a solid mixture were heated overnight at 100 °C, both browning and a characteristic malt-like odor were obvious. More recent studies designed to detect drug−excipient interactions generally rely upon the use of DSC[32] or diffuse reflectance FT-IR.[33] We chose to use the gradient HPLC method to directly analyze products from solid-state reactions.

To identify the important factors which would influence the solid state Maillard reaction of lactose and fluoxetine HCl, a series of screening experiments with various lactose types, mixing methods, water content, and lubricant concentrations were performed. The experiments are described in the experimental section and the results are summarized in Table 3. Four commonly available types of lactose were screened. Three mixing modes were chosen to mimic typical drug product unit operations including blending, milling, and wet granulation. Experiments with lactose monohydrate were also repeated with magnesium stearate added since this lubricant was commonly found in the generic lactose formulations. The relatively high temperature of 98 °C was chosen for expediency; the results are for comparison within the experiment, not for prediction of overall rates of decomposition of drug mixtures

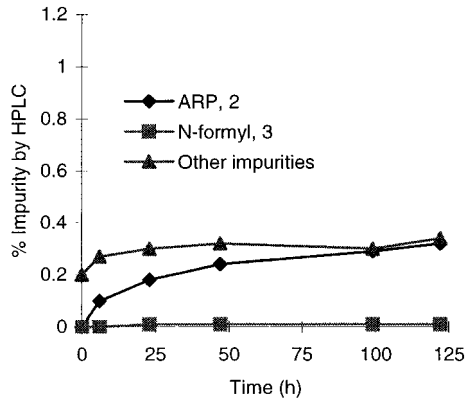


Figure 2—Lactose monohydrate and fluoxetine HCl at 85 °C.

under normal storage conditions. The gradient HPLC method was used to measure both the total amount of impurities as well as amounts of **2** and **3**.

The level of total impurities by HPLC in the bulk fluoxetine HCl used for this experiment was 0.23%; the level did not increase by heating fluoxetine HCl by itself and no significant impurities were observed by heating a lactose control individually. Analysis of replicates indicates the relative error in the impurity measurements to be somewhat high, about 20%. However, the wide range of results still allows for several statistically valid conclusions. The Maillard reaction was observed in all experiments and its rate was slightly faster with spray-dried lactose than with lactose monohydrate. Many products were detected by HPLC analysis, most of which were early-eluting carbohydrate-containing materials based on LC/MS analysis. Water accelerates the process. The rate of ARP formation with anhydrous and monohydrate lactose forms were similar, but the anhydrous form was less sensitive to added water, possibly due to conversion to lactose monohydrate. The rate was not significantly different in blended mixtures versus ground mixtures. The addition of magnesium stearate catalyzed the formylation process. On the basis of the pH-dependent data generated in solution, this effect may be due to localized changes in pH rather than changes in physical mobility within the solids due to the lubricant. This set of experiments clearly indicated that the Maillard reaction of fluoxetine HCl and lactose were likely to occur, even in the solid phase, and that factors such as moisture content, type of lactose, and the presence of magnesium stearate could have an influence on the rates and the identities of subsequent decomposition products.

The analysis of several lactose-containing generic fluoxetine HCl products by X-ray powder diffraction (XRPD) indicated that only lactose monohydrate (or spray-dried lactose) was being used; therefore, additional work with the anhydrous forms was not pursued. X-ray analysis was also used to qualitatively verify that even after heating for several days at 100 °C and in mixtures with substantial amounts of impurities, crystalline lactose monohydrate remained; anhydrous lactose was not detected, although some amorphous material could have been present. To examine the rates of this drug−excipient interaction in more detail and at a lower temperature, a series of kinetic experiments was performed as described in the experimental section. These conditions were chosen to be more representative of stress stability conditions typically used during drug development. The impurity content of the starting fluoxetine HCl was 0.20%. Representative results for 85 °C are displayed in Figures 2−4, which use the same vertical scale for ease of comparison.

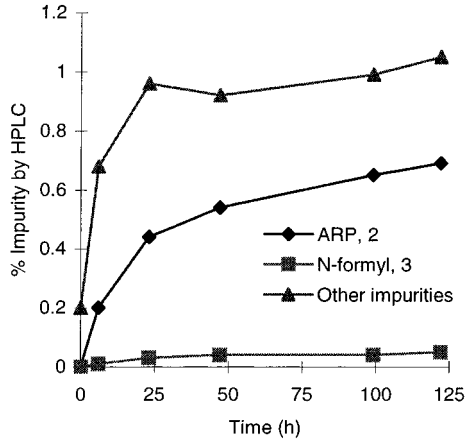


Figure 3—Spray-dried lactose and fluoxetine HCl at 85 °C.

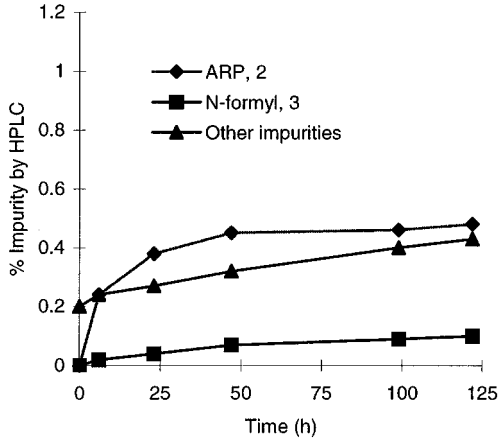


Figure 4—Lactose monohydrate, fluoxetine HCl, and magnesium stearate at 85 °C.

It is evident that in this study, substantially more ARP (**2**) was formed with spray-dried lactose (Figure 3) than when lactose monohydrate was used (Figure 2), consistent with previous reports.[13] In the former case, the total level of impurities exceeded 1% after just 1 day at 85 °C. Consistent with the screening study, the presence of magnesium stearate increased the amount of *N*-formylfluoxetine, **3**; compare Figures 4 and 2. Control experiments with isolated fluoxetine HCl and lactose were performed; both are stable under the conditions studied.

Temperature-dependent analysis of the results was performed using the simplest model for the production of an unstable product (eq 1). Product **X** refers to decomposition products of **2**, not any specific compound. The rates of formation of **2** and **X** were derived from eqs 2 and 3 in which T_r, the reference temperature, is 348 K. This modification was used to make the preexponential factor more independent of the activation energy, as suggested by Blau and co-workers in their description of Simusolv,[34] the simulation software in use. Only data for the concentration of **2** were used in the model since the identities of its degradation products, and thus their response factors, are largely unknown. Arrhenius parameters are summarized in Table 4.

$$\mathbf{1} \xrightarrow{k_1} \mathbf{2} \xrightarrow{k_2} \mathbf{X} \tag{1}$$

$$\frac{d[\mathbf{2}]}{dt} = k_1 \exp\left(\frac{E_1}{R}\left(\frac{1}{T_r} - \frac{1}{T}\right)\right)[1] - k_2 \exp\left(\frac{E_2}{R}\left(\frac{1}{T_r} - \frac{1}{T}\right)\right)[2] \tag{2}$$

$$\frac{d[\mathbf{X}]}{dt} = k_2 \exp\left(\frac{E_2}{R}\left(\frac{1}{T_r} - \frac{1}{T}\right)\right)[2] \tag{3}$$

The last line in Table 4, an extrapolation to the time required for the sum of **2** and its degradants, **X**, to total 0.1% at 30 °C, according to eqs 2 and 3, is included as an indication of the possible real-time magnitude of this drug–excipient interaction. The prediction that this process should be observable at ambient temperature is confirmed by the detection of ARP, **2**, in unstressed, unexpired lactose formulations; refer to Table 5. Figure 5 shows the experimental concentration of ARP, **2**, versus time for the mixture of lactose monohydrate, fluoxetine HCl, and magnesium stearate at all three temperatures and the curves generated from eq 2 using the parameter estimates given in Table 4. Due to the competitive decomposition of **2**, its concentration rises to a moderate level and remains fairly constant. That is, it is produced and consumed at approximately the same apparent rates. That it continues to be produced is indicated by continued growth in other impurity peaks, including *N*-formylfluoxetine, **3**.

Table 4—Arrhenius Parameters for Fluoxetine HCl and Lactose Thermolysis Based on Eqs 1–3[a]

parameter	monohydrate	spray-dried	monohydrate + Mg stearate
$k_1 \times 10^5$, 1/h	4.8(0.3)	6.9(0.9)	11.9(0.8)
$k_2 \times 10^2$, 1/h	2.4(0.2)	2.3(0.1)	4.0(0.3)
E_1, kcal/mol	24.9(1.1)	34.9(1.3)	26.8(1.3)
E_2, kcal/mol	13.4(1.8)	18.5(2.0)	16.6(1.5)
R^2 for model	0.97	0.96	0.98
months to **2** + **X** = 0.1% (30 °C)	5.9	34	3.5

[a] The standard deviation is in parentheses.

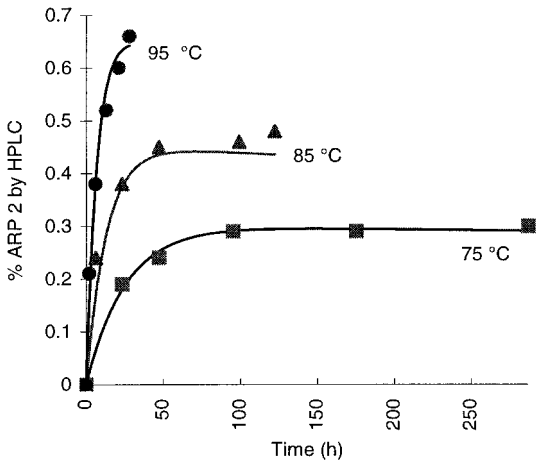


Figure 5—Formation of ARP, **2**, from solid-phase mixtures of lactose monohydrate, fluoxetine HCl, and magnesium stearate.. Points are experimental; lines are from eq 2.

Due to severe limitations on mobility, the kinetics of solid state systems, especially bimolecular ones, are not straightforward. Cohn utilized a function similar to the Arrhenius equation but which includes a parameter for the thickness of the reacting solid phases.[35] Carstensen has reviewed the problem as applied to the stability of solids and solid dosage forms and uses both the Arrhenius expression as well as Cohn's equation.[36] In the present study, the use of data from only the first few percent of the conversion minimizes the impact of these homogeneity issues, and the goodness of the fit to the model indicates that valid assumptions have been made.

The relevance of the above analysis to actual marketed generic fluoxetine HCl products was examined by replica-

Table 5—Stability of Fluoxetine HCl Products at 40 °C, 75% RH

product	impurity	% impurity by HPLC initial	1 mo	3 mo	6 mo	9 mo
Prozac	total	0.17	0.19	0.21	0.23	0.23
(starch)	**2**					
	3		0.01	0.01	0.01	0.02
generic A	total	0.43	0.47	0.60	0.90	1.10
(lactose)	**2**	0.03	0.01	0.01	0.05	0.05
	3		0.02	0.03	0.13	0.22
generic Z	total	0.30	0.35	0.45	0.63	0.74
(lactose)	**2**		0.01	0.02	0.03	0.02
	3		0.01	0.04	0.08	0.13

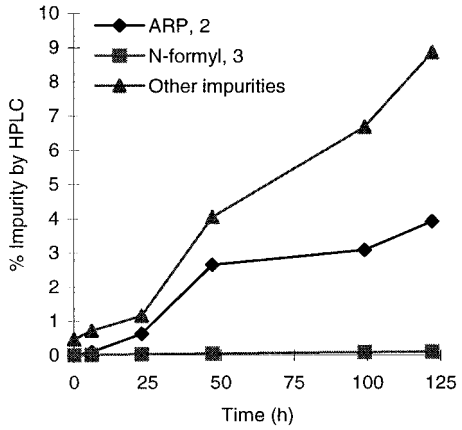


Figure 6—Generic N, a fluoxetine HCl/lactose capsule at 85 °C.

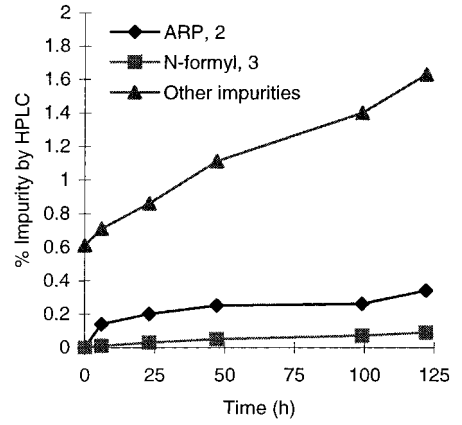


Figure 7—Generic O, a fluoxetine HCl/lactose tablet at 85 °C.

tion of the stress stability study with a capsule (generic N) and a tablet (generic O). The results are shown in Figures 6 and 7, respectively. Both products displayed Maillard browning and produced 2, 3, and many other impurities. The results from the tablet (Figure 7) are comparable with decomposition profiles of the simple mixtures of lactose, drug, and magnesium stearate. However, the capsule was significantly less stable, showing formation of nearly 4% of **2** after 122 h at 85 °C, and a total impurity level of 13%. This implies the presence of additional catalysts in this capsule, the identities of which are unknown.

The third type of stability study on lactose formulations of fluoxetine HCl utilized the more standard accelerated conditions of 40 °C and 75% relative humidity used to support regulatory submissions. In this study the gradient HPLC assay was used to assess the stability of Prozac and two generic lactose-containing capsules, designated generic A and generic Z. The results from 9 months are given in Table 5. Blanks indicate a level less than the detection limit of 0.01%.

Several points are of interest. First, the formulation with starch showed very limited impurity formation while the two formulations with lactose more than doubled in their total impurity content. Second, generic A (and other formulations examined) contain detectable amounts of **2** even before stressing, proving that the Maillard reaction occurs even at ambient temperature in unexpired products. Third, significant amounts of *N*-formylfluoxetine, **3**, was produced by stressing both lactose formulations and its growth rate in generic A was faster than in generic Z, which may be due to the higher concentration of magnesium stearate in the former. Finally, although formation of **2** is proof of the Maillard reaction, its concentration may not rise to a very high level due to its conversion to other products, including **3**. Thus, these formulations exhibited the same general behavior as noted in the solution phase experiments and the excipient interaction studies.

Solid-State Reactions Monitored by Headspace GC/MS—Although many of the products of the Maillard reaction are nonvolatile, such as the ARPs and melanoidins, many volatile products are produced as well. These have typically been identified by gas chromatography, especially by detection with a mass spectrometer. These studies frequently involve thermolysis of the ARP itself rather than the sugar−amine mixture. For example, Mills and Hodge studied the volatile products from pyrolysis of 1-deoxy-1-L-prolino-D-fructose,[37] Hayase and Kato studied the glucose−butylamine system,[27] Birch et al. examined 1-amino-1-deoxyfructoses derived from alkylamines,[38] and Shigematsu et al. has identified volatile products from pyrolysis from several ARPs derived from glucose and amino acids.[39] Although a diverse array of products has been identified, some compounds or classes of compounds are almost always observed. These include carboxylic acids (especially formic and acetic acid), acyclic carbonyl compounds such as aldehydes and ketones, dicarbonyl compounds such as 2,3-pentanedione, furans such as furan itself but especially 2-furaldehyde and furfuryl alcohol, other oxygen-containing heterocycles such as pyranones and furanones and their dihydro counterparts, amides of the amines (especially formamides and acetamides), and, in cases where a primary amine was used, pyrroles and other nitrogen-containing heterocycles.

To provide the link between the drug−excipient interaction under study and the cited chemistry (which was done mainly with monosaccharides and simple amines or amino acids), we have examined the volatile products of the solid-state reaction of lactose monohydrate and fluoxetine HCl by GC/MS as reported in the experimental section. The data from six such experiments is summarized in Table 6. These include lactose−fluoxetine HCl with and without added magnesium stearate (5% relative to lactose), two generic capsule formulations, and two positive controls with dimethylamine HCl and piperidine HCl. The top part of the table contains products derived from the disaccharide while the lower portion contains products derived from the amine. Major products are indicated by +++ and minor ones by a single +; the quantities are thus relative within an experiment and between experiments but were not accurately determined.

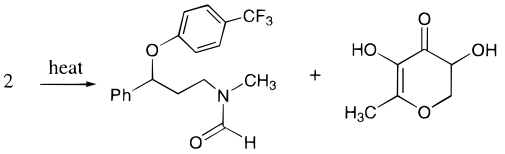
Three “negative" controls, lactose alone, fluoxetine HCl alone, and a Prozac capsule (starch-based formulation) were also performed. Essentially no decomposition was observed; none of the compounds in Table 6 were produced in these control experiments.

All of the products derived from the sugar have been observed by previous workers in the Maillard−Amadori arena, thus clearly linking the present study to their

Table 6—Volatile Products from Pyrolysis (100 °C, 20 h) of Lactose and Secondary Amine Hydrochlorides

compound	GC/MS retention (min)	fluox HCl + lactose	fluox HCl + lactose + Mg stearate	generic A	generic Z	Me_2NH HCl + lactose + Mg St	piperidine HCl + lactose + Mg St
vinyl acetate[a]	3.1		+	+	+	+	+
acetic acid[a]	3.8	+	++	+++	+++	+++	+++
2,3-pentanedione[a]	5.47		+	+	+		
4-methyl-3-pentenal	6.70			+	+		
3-furaldehyde[a]	8.04					++	++
2-furaldehyde[a]	8.43			+		+	+
2-furfuryl alcohol[a]	9.12		+			+	+
3-furfuryl alcohol[a]	9.36		+		+	+	+
2-acetylfuran[a]	10.21			+		+	
isomaltol	11.79			+		+	+
maltol[a]	14.19	+	++	++		+	
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**5**)[a]	14.79	+++		++		++	+
styrene[a]	10.12	+	+	+			
4-(trifluoromethyl)cresol[a]	13.45	+++	+++	+++	+++		
N-formylfluoxetine (**3**)[a]	29.34	+	+	+	+		
N,*N*-dimethylformamide[a]	7.09					++	
N,*N*-dimethylacetamide[a]	9.15					+	
N-formylpiperidine[a]	14.53						++
N-acetylpiperidine	15.74						+

[a] Matched both the mass spectrum from a library and the retention time of authentic compound.



N-formylfluoxetine, 3 5

Scheme 3—Decomposition of ARP, **2**.

findings. Of particular note is the widespread formation of 2-furaldehyde and other furans and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 5; see Scheme 3. The identity of this compound was proven by comparison with authentic material, prepared according to a published method.[40] Kim and Baltes recently reported that many cyclic and acyclic Maillard products, including several listed in Table 6, can be derived from thermolysis of this pyranone.[41] The possible presence of 1,2-dicarbonyl compounds (such as 2,3-pentanedione) in thermally stressed generic fluoxetine HCl products is of concern since some of them, including glyoxal and maltol, have been reported to be weak bacterial mutagens.[42]

It is also noteworthy that many of the same products were produced from the reactions with the HCl salts of dimethylamine and piperidine as with fluoxetine HCl. For example, the formamides of all three amines were found as well as furaldehyde and maltol. This proves that fluoxetine is not unique or unusual in this regard; nearly all secondary amines whether cyclic or acyclic, should undergo similar reactions with lactose, in agreement with the bulk of the scientific literature.

The results of the studies on solution-phase experiments, solid-phase excipient interaction screening, stability studies on fluoxetine HCl and lactose and formulated products, and the analysis of products clearly prove that the Maillard reaction occurs in this system. Although the clinical significance of this drug−excipient interaction is unknown, the relevance of these findings to formulation scientists are more straightforward. Namely, the Maillard reaction of secondary amines and lactose should be considered when selecting formulation ingredients and when examining the stability of such products. However, in many cases, the formulation scientist will not know whether a specific nitrogen-containing drug will be compatible with reducing carbohydrates or not, usually due to significant structural variations such as inclusion of the nitrogen within rings or the presence of functionality which would greatly diminish the nucleophilicity of the alkaloid drug. The present findings suggest a relatively simple experimental design to probe this question, namely, the use of the formamide derivative as a chemical marker for the Maillard reaction. In all systems studied here (and most in the literature in which their presence would have been detected) *N*-formyl compounds have been observed as a major product of the Maillard process. Therefore, simple reaction of an alkaloid drug with acetic formic anhydride should provide samples of *N*-formyl compound(s) which may or may not be isolated and purified, but which are usually stable and usually separable from the drug on chromatographic systems such as GC, HPLC, or electrophoresis. These derivatives will often be readily available and detectable and their formation in stressed samples of potential formulation mixtures will be a strong indication of the Maillard reaction. This chemical marker will be more accessible than Amadori rearrangement products since the latter are often unstable and are difficult to prepare, isolate, and characterize. The more traditional method of screening simply for color and odor is still possible; however, these sensory clues are not present in early stages of the Maillard process when impurity levels may exceed the level of 0.1%.[18] An alternative screening program for the presence of Maillard-based drug−excipient interactions using GC/MS detection of volatile carbohydrate-derived compounds such as given in Table 6 could also be useful.

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