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Research Article

Assessment of Feasibility of Maillard Reaction between Baclofen and Lactose by Liquid Chromatography and Tandem Mass Spectrometry, Application to Pre Formulation Studies

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Abstract. The aim of this study was to determine any possible, baclofen–lactose Maillard reaction products. Granules and tablets of baclofen and lactose were prepared and maintained in heat ovens for a certain time period. The effects of lactose type, addition of magnesium stearate, and water were monitored. Heated lactose and baclofen were analyzed using reverse-phase HPLC. Liquid chromatography tandem mass spectroscopy revealed nominal mass values consistent with baclofen–lactose, early-stage Maillard reaction condensation products (ESMRP). Multiple reaction monitoring confirmed the presence of ESMRP as well. FTIR analysis proved the formation of imine bond. The results indicated that baclofen undergoes a Maillard-type reaction with lactose.

KEY WORDS: baclofen; lactose; LC-MS/MS; Maillard reaction; solid-state incompatibility.

INTRODUCTION

Maillard reaction is named after Louis Maillard, who reported over 95 years ago that some amines and reducing carbohydrates react to produce brown pigments (1). The reaction is actually a series of complex reactions between reducing sugars such as D-glucose and free amino groups of amino acids, peptides, or proteins (2,3). The mechanism of the Maillard reaction is very complicated; however, it is generally divided into three stages (4):

(1) The first stage involves the sugar-amine condensation and the Amadori rearrangement. The reaction steps have been well-defined and no browning occurs at this stage
(2) The second stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker reaction especially at high temperatures.
(3) Formation of heterocyclic nitrogen compounds. Browning occurs at this stage.

Generally, the first product of this reaction is a simple glycosylamine (5), which readily undergoes the Amadori rearrangement to produce 1-amino-1-deoxy-2-ketoses (6). The later stages are complex and variable, depending on the reaction conditions, and involve further dehydration and fission of the initial reaction products (7). Melanoidines are the final products of the reaction and are actually brown nitrogen containing polymeric substances that decompose with difficulty (8,9). Browning is usually measured spectrophotometrically (at 490 nm) and expressed in absorbance units (8).

Maillard reaction has been extensively studied and reviewed, especially in the food- and nutrition-related literature and it is a well-documented process for the degradation of lactose during the heating of milk (3,10–14). Nowadays, mass spectrometry (MS) has been adopted by many researchers attempting to reveal the details of Maillard chemistry (9,15–18). Whereas traditional mass analysis would provide the fragment ions resulting from all compounds contained within a sample, tandem mass spectrometry provides information regarding the fragment ions that originate from a single molecular ion (9). Mass spectroscopy along with a separation technique such as HPLC results in an extremely powerful analytical technique (9). Fay *et al.* demonstrated the benefits of tandem MS in their study of a pentose–glycine reaction system (19).

Among pharmaceutical excipients, lactose, a reducing sugar, is widely used as a filler in tablet and capsule formulations (20). A survey of the Physician's Desk Reference database shows that there are many pharmaceutical formula-

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tions where amino compounds and lactose are both present (21,22). In formulations, solid-state reactions of the drug substance are of great interest, especially in cases where the drug substance is intrinsically and chemically reactive or unstable (23). Recently, the possible reaction of amine groups of drug entities with carbonyl groups of common tablet excipients, such as lactose, starch, and cellulose has gained a pharmaceutical interest (7,18,22–26). Baclofen is an amine-containing skeletal muscle relaxant, which is used to relieve the signs and symptoms of spasticity due to spinal cord injury or multiple sclerosis (27). It is a zwitterion containing both carboxylic and amine moiety in its structure, which makes it a good candidate to react with a reducing sugar such as lactose.

Solid-phase chemical reaction is dependent on physical contact between solid components. It has been shown that extensive solid-state reactions can occur after tableting of pharmaceutical formulations which is due to increased contact between reactants.

Although the chemistry of the Maillard reaction is well known, detailed data using mass spectroscopy in pharmaceutical technology are limited and needs more examples with detailed mechanism of the reaction to show that the reducing sugars should be avoided in formulating of amine-containing drugs in the pharmaceutical industry.

Recently, Cutrignelli *et al*. studied the comparative effects of some hydrophilic excipients on the rate of gabapentin and baclofen lactamization in lyophilized formulations. They suggested that a possible gabapentin/lactose (not baclofen/lactose) Maillard-type interaction results in a moderate increase in lactam formation (28). This study was generally about the rate and extent of baclofen and gabapentin lactamization. To the best of our knowledge, evaluation of the Maillard reaction products of baclofen and lactose has not been investigated yet. In the present study, different mixtures of the drug and excipient have been studied by HPLC, FTIR, visible spectrophotometry, and tandem mass spectroscopy to determine the possible early-stage Maillard reaction condensation products and final stage brown color. The effect of lactose type, temperature conditions, and the presence of magnesium stearate has been studied as well.

The other novelty of this study is that not only the HPLC, mass analysis, and FTIR is done to prove the possible Maillard reaction between the drug and excipients but also visible spectrophotometry as a less sophisticated method of evaluating the Maillard reaction which was widely used in the past, has been investigated simultaneously to show that still, with some considerations, spectroscopic assessment of the observed browning phenomena is a remarkable tool to evaluate this type of reaction in pharmaceutical sciences. This study also introduces a new stability-indicating HPLC method with regard to baclofen/lactose Maillard reaction products, using internal standard.

MATERIALS AND METHODS

Materials

Baclofen (*R*,*S*-Amino-3-(4′-chlorophenyl) butyric acid) was obtained from Pfizer-groups, Istanbul, Turkey. Lactose monohydrate (Pharma grade 200 Mesh) and anhydrous lactose provided from DMV Chemical Co, Netherlands. All chemicals were of HPLC or analytical grade obtained from LAB scan analytical science, Ireland. Commercial tablets of baclofen (Zahravi, Kimidaroo, and Clofen) were purchased directly from Iran and Australia.

Methods

Analytical Methods

HPLC. The HPLC system consisted of a SCL-10A XL auto injector, SCL-10A VP system controller, LC-10AT liquid chromatograph and a SPD-M10AVP, UV–VIS, photo diode array (PDA) detector and a FRC-10A fraction collector, all from Shimadzu (Kyoto, Japan).

Samples were injected onto a Hypersil C18-BDS column (100 mm, 4.60 mm, 5 μm; Phenomenex, Torrance, CA) maintained at ambient temperature. Mobile phase was 13% acetonitrile in phosphate buffer 25 mM and pH was adjusted to 9.3, using sodium hydroxide. Flow rate was 1 mL/min with detection at 220 nm. Data were analyzed with Class VP software (version, 6.14 SP1). A solution of 3-methyl salicylic acid (0.5 mg/mL in mobile phase) was used as the internal standard. Internal standard solution (10 μL) was added to each experimental sample (100 μL). The analytical method was validated with respect to parameters such as linearity, intermediate precision, accuracy, and selectivity (29,30).

LC-MS/MS. The LC system consisted of a SIL-10AD VP auto injector, SCL-10A VP system controller, LC-10ADVP liquid chromatograph and a DGU-12A degasser, all from Shimadzu (Kyoto, Japan).

Samples were introduced into the mass spectrometer through a C18 Gemini column (2×5×200 mm, Phenomenex) eluted at a flow rate of 0.2 mL/min, at ambient temperature. Elution was performed, with 97% solvent A (0.1% formic acid in water) and 3% solvent B (0.1% formic acid, 90% acetonitrile, 5% methanol, and 5% water). Mass spectrometric detection was performed with an Applied Biosystems MDS Sciex (Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface in the positive ion mode. The tandem mass spectrometer was operated at unit resolution in the multiple reactions monitoring mode (MRM), monitoring the transition of the protonated molecular ions to the product ions. Q1 was used from 150–600 amu in a mass-resolving mode to select the parent ion. The ion source temperature was maintained at 350°C. The ionspray voltage was set at 5,500 V. The curtain gas (CUR; nitrogen) was set at 15 and the collision gas (CAD) at 7. The collision energy (CE), declustering potential (DP), focusing potential (FP) and entrance potential (EP), were set at 40, 75, 200, and 8 V, respectively. This system was set to the multiple reaction monitoring (MRM) mode, that is, selecting precursor ions, dissociating them and finally analyzing the product ions reaching the high selectivity and sensitivity of this mode for mass analysis and detection. In the MRM mode, data acquisition and processing were accomplished using the Applied Biosystems Analyst version 1.4.1 software.

Spectrophotometer. As mentioned before, brown polymers are formed at the final stages of the Maillard reaction (4,13,31). In order to determine the intensity of brown color samples were monitored spectrophotometrically using a plate reader (Spectra Count Packard, Meriden, USA) in a Nunc® 96-well-plate with 490 nm filter and the optical density (OD) was recorded.

FTIR. Infrared spectra were recorded on a Bomem MB-100 spectrometer (Bomem, Quebec, Canada), in the range of 400–4,000 cm^{-1}, using KBr disks. Resolution was 4 cm^{-1}, 37 scans/min. The spectrum was a mean of ten consecutive scans on the same sample. Processing of the FTIR data was performed using GRAMS/32 version 3.04 (Galactic Industries Corporation, Salem, NH).

Formulation Methods

Preparation of baclofen–lactose adduct mixture. Baclofen (0.5 g) and lactose monohydrate (2.5 g) were dissolved in 50 mL of United States Pharmacopoeia (USP) borate buffer (0.1 M, pH=9.2) with the aid of stirring and ultrasound (30). The ionic strength of the solution was adjusted to 25 mM by sodium chloride. Triethylamine was added in an equimolar ratio with baclofen to aid solubility. The clear solution was then refluxed at 60°C using a water bath (Contherm Scientific Ltd, New Zealand) for 12 h and dried overnight at the same temperature in an open Pyrex™ beaker using a heat oven. The dried mixture is referred to as the adduct mixture. Adduct mixtures were dissolved in mobile phase to get 1 mg/mL concentration with respect to the baclofen and was subjected to reversed-phase chromatography and LC-MS/MS. The intensity of the brown color was also measured at 490 nm. Different samples of baclofen (solid-state solution, pH=9.2), aqueous mixture of baclofen and lactose (pH=9.2) and commercial tablets were heated in order to yield degradation products. All solid and liquid samples were heated in 90°C ovens and 60°C water bath for 24 and 72 h, respectively.

Tablet preparation. Baclofen and lactose either anhydrous or monohydrate were mixed in 1:1, 1:5, and 1:10 *w/w* ratios. Binary mixtures were subjected to dry granulation by slug preparation technique. Granules were directly compressed to tablets and were kept in silica-gel-containing desiccators for 10 days. Dry samples were kept in well-closed containers. The containers were placed inside silica gel chambers and maintained at 25, 40, 50, and 60°C in ovens for a 6-month period. The relative humidity in the chambers was kept at almost zero which was confirmed by a hygrometer (Lutron HD-3008 Hygrometer, Taiwan). Each sample was dissolved in mobile phase and analyzed using HPLC DAD (diode array detection), LC-MS/MS, and FTIR. The intensity of the brown color was also measured spectrophotometrically (at 490 nm).

Commercial tablets and screening tests. The Serajuddin (32) method with minor modifications was employed to monitor the probable solid-state interaction of drug with excipient in an accelerated manner. Briefly, drug powders with 20% added water was kept in well-closed 4-mL HPLC grade glass vials at 95°C for 12 h. The total weight of the drug:excipient blend in a vial was kept at 200 mg for each of the three brand formulations. Controls were done using the

Table I. Composition and Assay Results of Screening Samples

	Composition					Assay		
Samples[a]	Baclofen	Mg stearate	Lactose anhydrous	lactose monohydrate	water	Baclofen (%)	Unknown-1	OD
1	+[b]	−[c]	+	−	−	83.96	0.08	0
2	+	−	+	−	+	1.34	1.03	0.191
3	+	+	+	−	−	92.21	0.11	0.016
4	+	+	+	−	+	1.15	0.24	0.212
5	+	−	−	+	−	82.75	0.49	0.015
6	+	−	−	+	+	1.21	0.45	0.192
7	+	+	−	+	−	90.77	0.58	0.016
8	+	+	−	+	+	2.11	0.51	0.233
9	+	−	−	−	−	89.39	0	0
10	+	−	−	−	+	76.24	0	0
11	Brand 1	−	−	−	−	91.79	0.73	0.01
12	Brand 1	−	−	−	+	0.49	0.08	0.43
13	Brand 2	−	−	−	−	80.09	0.46	0.00
14	Brand 2	−	−	−	+	0.55	0.18	0.41
15	Brand 3	−	−	−	−	77.42	0.31	0.07
16	Brand 3	−	−	−	+	0.83	1.18	0.50
17	−	−	+	−	−	−	−	0
18	−	−	+	−	+	−	−	0
19	−	−	−	+	−	−	−	0
20	−	−	−	+	−	−	−	0

[a] Physical mixtures of the contents
[b] Presence
[c] Absence

Table II. Intermediate Precision and Accuracy of the HPLC Method

Actual concentration (μg/ml)	Measured concentration (mg/ml), RSD (%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
62.5	0.0614, 1	0.616, 0.6	98.24	98.56
125	0.126, 2	0.124, 1.9	100.8	99.2
250	0.248, 1.4	0.258, 2.1	99.2	103.2
500	0.49, 0.9	0.512, 1.2	98.0	102.4

same procedure without lactose. The composition of the screening samples is listed in Table I. Samples were analyzed using HPLC to calculate the amount of the remaining drug. The brown color was measured spectrophotometrically at 490 nm.

Screening tests were conducted to examine the effect of lactose type (monohydrated or anhydrous), addition of water and magnesium stearate as a widely used lubricant. Mixtures were prepared according to Table I and kept at 95°C for 12 h. Samples were analyzed to determine the amount of remaining baclofen, possible Maillard reaction adduct/s and brown color

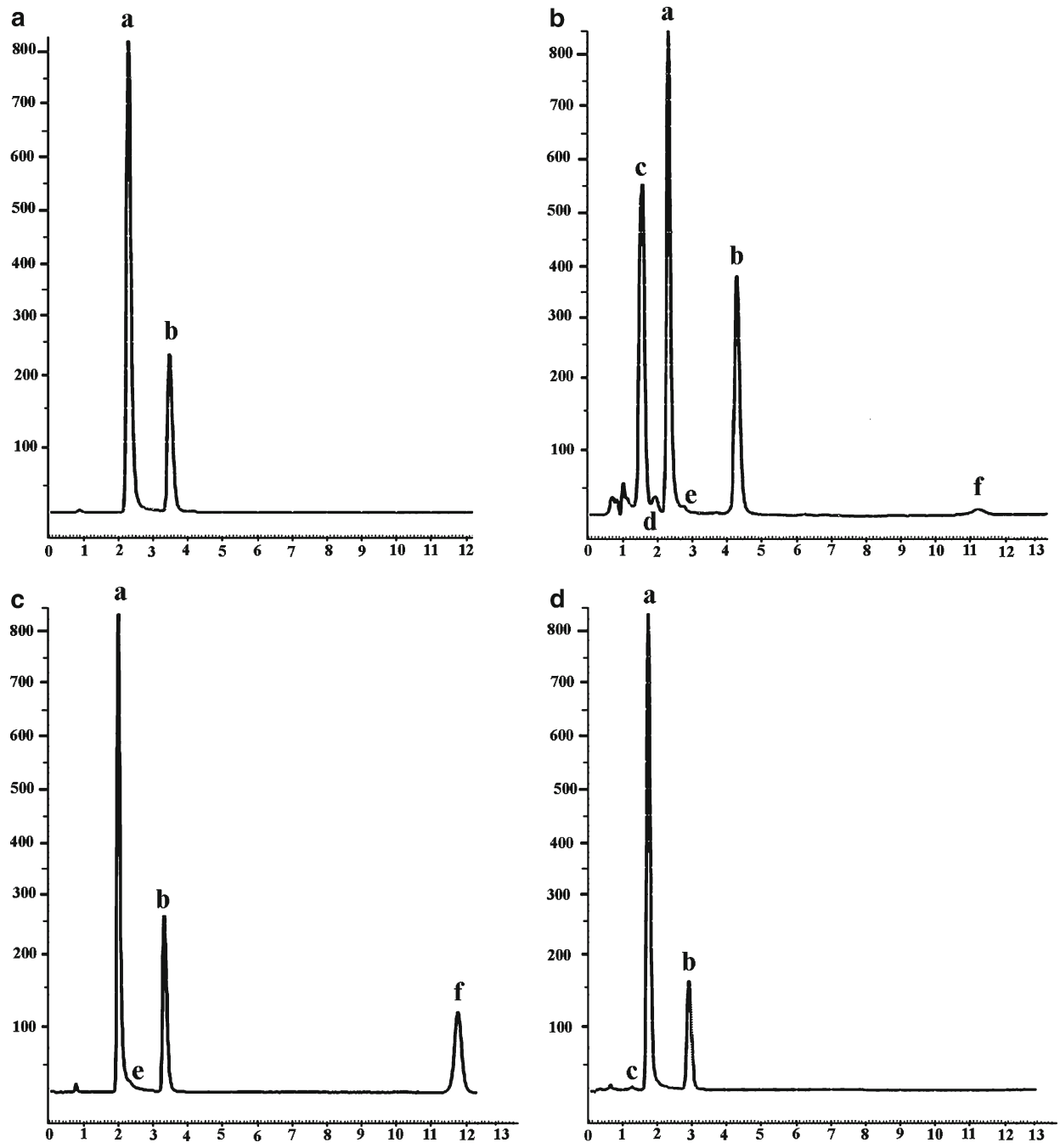

Fig. 1. HPLC chromatogram of **a** baclofen, **b** adducts mixture (heated baclofen and lactose), **c** heated baclofen in aqueous media, **d** intact brand 3. (*A*) baclofen, (*B*) internal standard, (*C*) unknown-l, (*D*) unknown-2, (*E*) unknown-3, (*F*) unknown-4. Note: in **a**, **b**, and **c** flow rate is 1 but in **d** flow rate 2

formation according to specified methods. Each experiment was done in duplicate.

The presence of lactose in selected formulations was examined according to the British Pharmacopoeia (BP), that is heating of a mixture of lactose (equivalent to 0.25 mg) with added ammonia (5 mL) and water (5 mL). Development of red color confirms the presence of lactose in the formulations (33). Twenty tablets of three different brands were finely powdered and assayed according to the United States Pharmacopoeia (USP) and then were kept at 95°C for 24 h in dry conditions.

RESULTS AND DISSCUSION

Analytical Methods

HPLC

HPLC method validation. The standard solutions for linearity test were prepared five times at different concentration levels. Peak area ratios of baclofen to internal standard were calculated and plotted *versus* respective concentrations and linear regression analysis performed. The constructed calibration curve was linear over the concentration range of 7.8–1,000 μg/mL. Correlation coefficient was found to be more than 0.996 with relative standard deviation (RSD) values ranging from 0.16–3.03% within the concentration ranges studied. Repeatability of measurements of peak area was carried out using seven replicates of the same concentration (62.5 μg/mL). The RSD was found to be 1.2%.

The intra- and inter-day precision of the method was carried out at four different concentrations (62.5, 125, 250, and 500 μg/mL). The low RSD values of within-day and day-to-day variations revealed that the proposed method is precise (Table II).

Limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios using an analytical response of three and ten times the background noise, respectively (30). The LOD and LOQ were found to be 0.9 and 3 μg/mL, respectively. Selectivity of the method was tested using heated samples of baclofen with or without lactose. Chromatograms are presented in Fig. 1. Some useful standard chromatographic parameters have been calculated and reported in Table III.

Table III. Some Standard Chromatographic Parameters

Peak name	Retention time	Selectivity factor[a]	Resolution[b]
Solvent front	0.10	–	–
Internal standard	4.10	–	–
a–b	2.30	2.38[a]	3.64[a]
c–b	1.60	5.17[a]	2.70[a]
d–b	2.00	3.10[a]	3.23[a]
e–b	2.70	1.82[a]	5.86[a]
f–b	11.20	3.29[a]	6.17[a]
c–d		1.67[b]	1.07[b]
d–a		1.30[c]	0.55[c]
a–e		1.31[d]	0.67[d]

[a] Compared to internal standard
[b] Peak c compared to peak d
[c] Peak d compared to peak a
[d] Peak a compared to peak e

HPLC analysis of the adduct mixtures. Each adduct mixture was dissolved in mobile phase to produce a solution with a nominal baclofen concentration of 500 μg/mL. In comparison to initial solution HPLC analysis of the baclofen–lactose adduct mixture revealed extra peaks (labeled as c, d, e, and f) in addition to baclofen and the internal standard (Fig. 1a and b). These extra peaks were named as unknown 1–4. Baclofen elutes at 2.3 min, whereas the internal standard, elutes at 4.1 min.

Heated baclofen showed two peaks (labeled as e and f) and named unknown-3 and unknown-4 respectively (Fig. 1c). Two major unknowns eluted before baclofen indicated as unknown-1 and unknown 2 in Fig. 1b. These species are more polar than baclofen but have UV spectra very similar to it, with maxima near 220 nm. Lactose samples either anhydrous or hydrous that were heated alone showed no extra peaks in the same chromatographic conditions compared to that obtained with the mixture.

Mass Spectroscopy

The same solution injected to HPLC was examined by LC-MS. A mass spectrometer compatible (salt-free) gradient method was developed to give similar separation to the HPLC method. Mass spectra (MS2) are presented in Fig. 2a–f. The full-scan positive ion electrospray product ion mass spectra showed that the precursor ions of baclofen and unknown-(1–4) were the protonated molecule, $[M+H]^+$, of *m/z* 214.7, 538.3, 376.2, 242.4, and 196.2, respectively (Fig. 2a–e). After collision-induced dissociation, the characteristic ions in the product ion mass spectrum were at *m/z* 151.2, 358.2, 358, 186.3, and 151.1, respectively. Proposed structures for unknown-1–4 are shown in Figs. 3 and 4, respectively. The nominal molecular mass of unknown-1 is consistent with the baclofen–lactose condensation product formed by the elimination of a single molecule of water from the parent compounds (Figs. 2b and 3a). According to Fig. 2c, unknown-2 indicates another condensation product of 376 amu which is related to baclofen–galactose or baclofen–glucose adducts (Fig. 3b). Galactose and glucose may be produced from lactose hydrolysis in aqueous solutions and high temperatures. According to BP (33) there are two major impurities in baclofen powder, one of them is an oxopentanoic acid derivative: (3*R*,*S*)-5-amino-3-(4-chlorophenyl)-5-oxopentanoic acid (CAS: 1141-23-7) and the other one contains a lactam ring and is named (4*R*,*S*)-4-(4-chlorophenyl) pyrrolidin-2-one (CAS: 22518-27-0; Fig. 4). The nominal mass values of these impurities are 195.6 and 241.7, respectively. It should be noted that unknown-3 and -4 shown in Fig. 1 (eluting after baclofen) have shown masses of 242.4 and 196.2 amu, respectively (Fig. 2d and e). This result corresponds to protonated molecule, $[M+H]^+$ of baclofen major impurities. The MRM mode was set for baclofen (214.7/151.2), unknown-1 (538.3/358.2) and unknown-2 (376.2/358). MRM chromatogram shown in Fig. 2f indicates the presence of 538 and 376 masses before baclofen in LC-MS/MS system as well. Manually collected HPLC fractions of unknown-1, unknown-

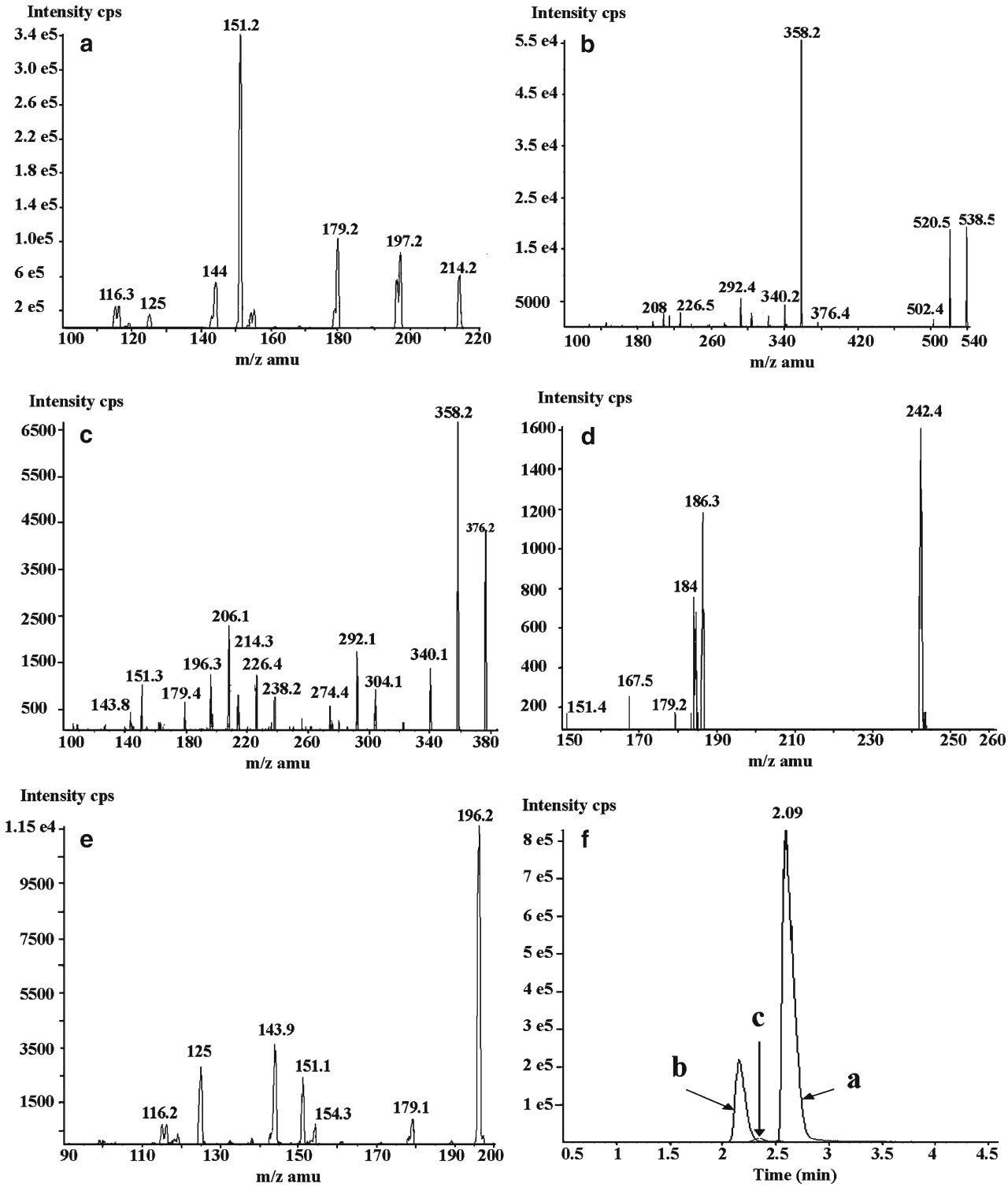

Fig. 2. Positive ion mode electrospray mass spectrum of **a** baclofen, **b** unknown-1, **c** unknown-2, **d** unknown-3, **e** unknown-4, **f** MRM chromatogram of three pairs; (*A*) baclofen, 214.1/151.2 amu, (*B*) unknown-1, 538.3/358.3 amu, and (*C*) unknown-2, 376.2/358.2 amu

2 were correlated by LC-MS/MS analysis. The unknown-1 and unknown-2 from Fig. 1 also correlated to the LC-MS/MS chromatograms by similar relative peak areas.

Spectrophotometry

Calculated absorbances of the samples have been presented in Table IV and are discussed in the "Formulation Methods" section under "Analysis of Prepared Granules and Tablets" and "Screening tests and Commercial Tablets" subsections.

FTIR Spectroscopy

As mentioned before, the first step of the Maillard reaction leads to the formation of an imine known as a Schiff's base. In Fig. 5, proposed changes in infra-red

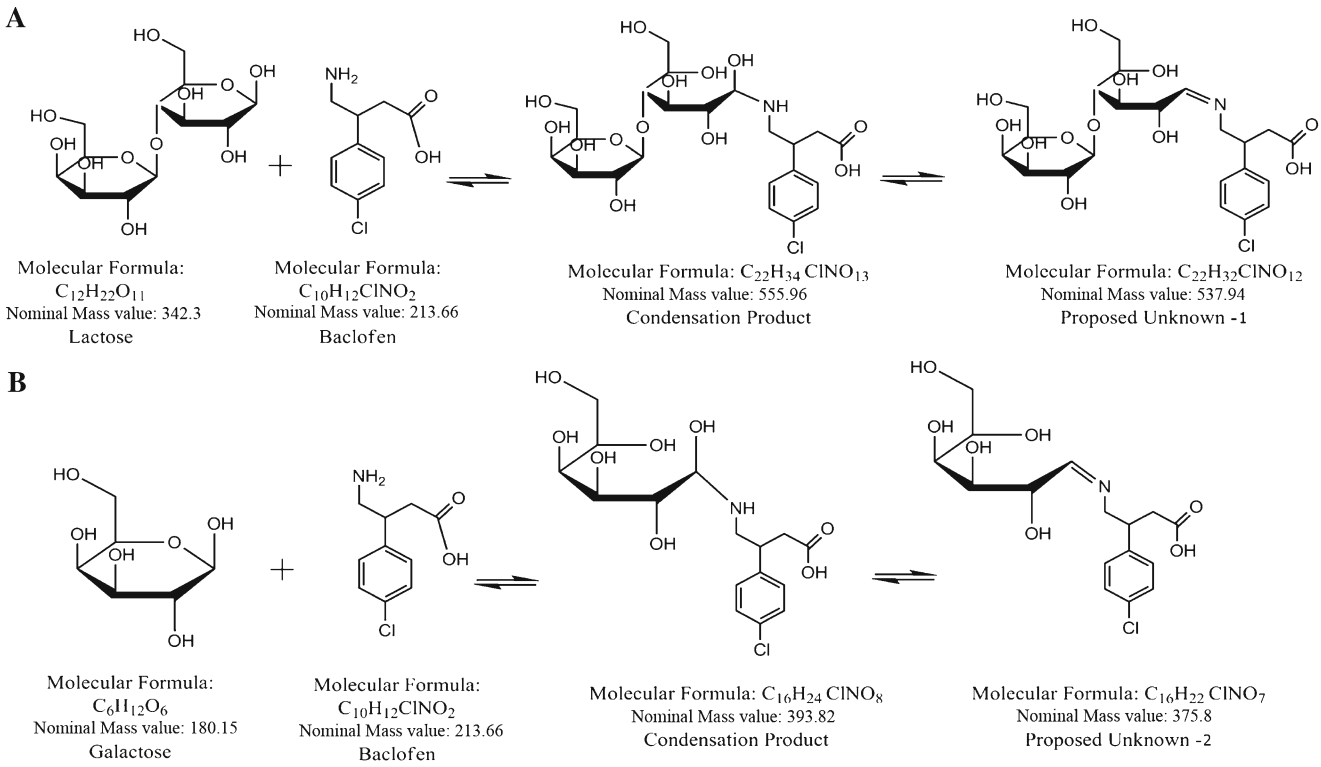

Fig. 3. Proposed Maillard reaction products for baclofen and lactose/glucose/galactose

absorption due to Maillard reaction is presented (34,35). The C=N stretching band appears at 1,630–1,650 cm^{-1} in the infrared spectra of imine containing compounds (35,36). Wnorowski *et al*. monitored carbonyl-amine reaction between pyruvic acid and α-amino alcohols by FTIR spectroscopy and reported that the resultant Schiff's base absorbs infrared light at about 1,647 cm^{-1} (35). Later at 2006, Namli *et al*. followed the reaction process between benzaldehyde or salicylaldehyde and or 2-pyridinecarboxaldehyde with aniline and showed that resulting imine, shows a band in FTIR spectra at about 1,630 cm^{-1} (36). Other scientists have reported imine formation using FTIR spectroscopy with similar absorption bands (37,38). In the baclofen FTIR spectrum, the absorption band at about 1618 cm^{-1} is consistent with baclofen N–H bending vibration (Fig. 6b). Adduct mixtures showed another absorption band around 1,648 cm^{-1} which is related to C=N stretching (Fig. 6a). The possibility that this imine is converted into its isomeric enamine form during the Maillard reaction has been proposed by Holtermand and has been referred to as a transamination reaction (35,39). The absorption pattern of baclofen–lactose 1:5 *w/w* after mixing and incubation at room temperature or 60°C for 6 months is shown in Fig. 6c, d, and e, respectively. Clearly the N=H bending vibration and C=N stretching absorption have appeared at 1,616 and 1,630 cm^{-1}, respectively. In the Fig. 6e an extra band at 1,666 cm^{-1} could be ascribed to the transamination process described in Fig. 5.

Formulation Methods

Analysis of Prepared Granules and Tablets

Because the degradation products are not available as reference materials or not known, their percentages in the solid degradation products were calculated based on the assumption that the HPLC detector's response factors for baclofen and the degradation products are the same. This kind of assumption has been previously made by Abdoh *et al.* in the assessment of amlodipine besylate and excipients interaction in solid dosage form (7).

Visual changes in heated tablets are presented in Fig. 7. The granules and the tablets were analyzed to determine the amount of remaining baclofen and unknown-1. Intensity of brown color was also measured. Results are presented in Table IV.

A closer look at Table IV reveals that in granules after 6 months incubation at different temperature conditions, the amount of remaining baclofen in the preparations containing anhydrous lactose is always more than the hydrated form which is in contrast with tablets. Although the first stage of Maillard reaction involves the removal of water molecule, but according to Serajuddin (32) and Abdoh (7) the presence of

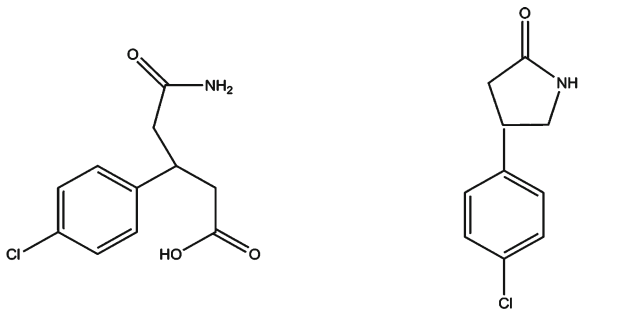

Fig. 4. Baclofen major impurities according to BP

some water promotes the Maillard reaction. This is in accordance with our observation, that the baclofen remaining in heated granules of monohydrated lactose is less than in granules prepared with anhydrous lactose. But in tablets, the trend is reversed. In granules, the physical contact between the chemical reactants is more limited than in tablets which have been compressed. Recent findings of Buisignies *et al.* indicates that lactose pseudopolymorphism seems to affect the compressibility more than anomerization or partial amorphization (40,41). It can be concluded that tablets prepared with anhydrous lactose will be harder than those prepared with monohydrate lactose. Stronger compaction of anhydrous lactose leads to better particle interlocking and enabling effective physical contact between the drug and excipient. Thus, the most important factor affecting the Maillard reaction in prepared tablets and granules are physical contact and moisture content, respectively.

Screening Tests and Commercial Tablets

Red color appearance in the lactose identification test, confirmed the presence of lactose in the brand formulations studied. The relatively high temperature of 95°C was chosen for expediency; the results are for comparison within the experiment, not for prediction of overall rates of decomposition of drug mixtures under normal storage conditions, as have been previously utilized by Wirth *et al.* in the study of fluoxetine incompatibility with lactose (26). Results of the screening test are presented in Table I. As it was expected (7,32), the presence of water promoted the degradation reactions including the Maillard reaction as the brown color develops more significantly in water-containing mixtures (samples 2, 4, 6, and 8).

In fast screening samples, the results with anhydrous and monohydrate lactose were similar. These results could be explained by the fact that high temperature forces the reaction to the end point but the long-term studies which were conducted at lower temperatures are at an intermediate stage of reaction progress. Therefore, it can be assumed that the rate of reaction with anhydrous and monohydrated lactose may be different, but at the end of the reaction, the amount of baclofen degradation is almost the same.

Wirth *et al.* showed that the addition of magnesium stearate catalyzed the formylation process between fluoxetine and lactose. They concluded that this may be due to localized changes in pH rather than changes in physical mobility within the solids due to the lubricant (26). In another investigation, Abdoh *et al.* demonstrated that catalytic effect of magnesium in the Maillard reaction of amlodipine besylate and lactose is attributed to local pH changes (7). According to Table I, except sample 4, all other magnesium-stearate-containing samples (3, 7, and 8), showed less baclofen loss in comparison to those prepared without magnesium stearate (1, 5, and 6). This may be due to a lubricant effect of magnesium stearate rather than its basic nature which prevents the effective contact between drug and excipient particles. However, more study is needed to establish the difference seen here compared to previous reports (7,26).

The amount of unknown-1 (ESMRP) simply indicates the occurrence of a reaction and cannot be considered as an indicator of reaction promotion because the intermediate

Table IV. Percentage of Unknown-1 and Remaining Baclofen and the Absorbance at 490 nm (optical density) in Tablets and Granules after Incubation at Different Temperatures for 6 months

		25°C				40°C				50°C				60°C			
		Granule		Tablet		Granule		Tablet		Granule		Tablet		Granule		Tablet	
		M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
1[a]	Remaining Baclofen (%)[b]	60.19	80.63	93.98	89.38	59.71	79.08	91.99	90.10	50.11	51.00	92.23	89.72	45.22	53.68	74.01	67.06
	Unknown-1(%)	0.19	0.26	0.25	0.32	0.28	0.27	0.22	0.33	0.38	0.24	0.25	0.35	0.41	0.23	0.28	0.39
	OD	0.001	0.002	0.014	0.001	0.001	0.003	0.000	0.002	0.001	0.002	0.000	0.002	0.008	0.013	0.014	0.016
5[c]	Remaining Baclofen (%)	97.96	98.18	98.38	97.02	96.35	98.68	92.37	90.23	90.06	94.76	95.87	90.22	72.15	75.88	82.84	78.23
	Unknown-1(%)	0.38	0.53	0.67	0.62	0.54	0.55	0.40	0.52	0.50	0.55	0.48	0.50	0.38	0.36	0.37	0.24
	OD	0.002	0.003	0.002	0.002	0.003	0.004	0.007	0.009	0.007	0.003	0.005	0.003	0.007	0.015	0.014	0.016
10[d]	Remaining Baclofen (%)	96.98	97.36	99.65	95.89	94.24	97.98	94.00	92.76	92.05	96.43	92.55	90.26	84.40	87.49	83.9	80.1
	Unknown-1(%)	2.12	2.09	2.39	2.26	2.21	2.29	2.33	1.67	2.11	1.83	2.19	2.20	1.79	2.05	1.42	1.64
	OD	0.001	0.002	0.003	0.002	0.004	0.003	0.005	0.005	0.006	0.003	0.002	0.007	0.009	0.014	0.013	0.015

M lactose monohydrate, *A* lactose anhydrated
[a] Baclofen:lactose 1:1 *w/w*
[b] RSD for all the experiments is between 0.3–3.1%
[c] Baclofen:lactose 1:5 *w/w*
[d] Baclofen:lactose 1:10 *w/w*

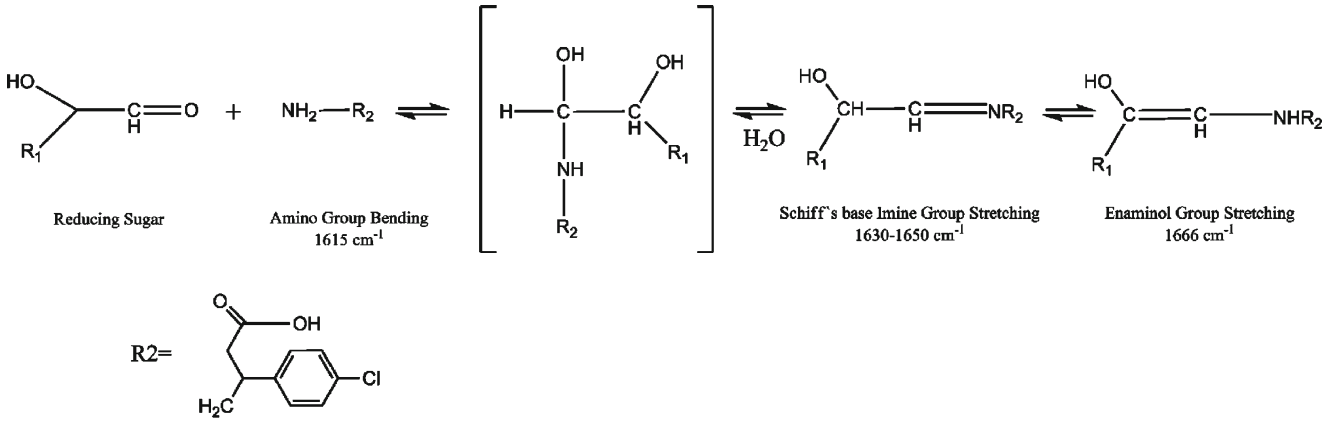

Fig. 5. Maillard reaction with FTIR peak changes. Adopted from Yates *et al.* with minor modifications (35)

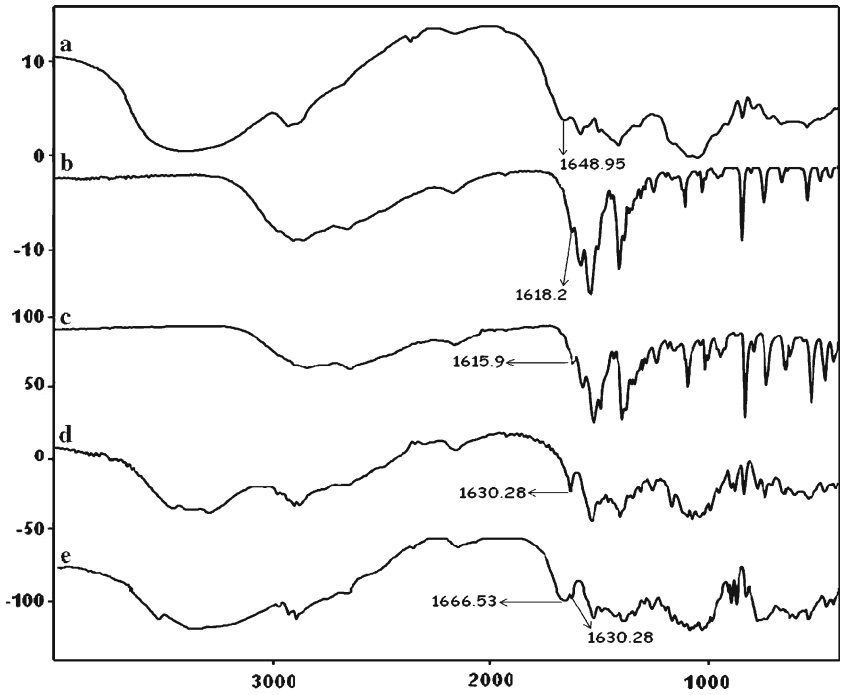

Fig. 6. FTIR spectra of (*A*) adduct mixture, (*B*) baclofen, (*C*) baclofen:lactose monohydrate (1:5 *w/w*) after mixing, (*D*) sample *C* after 6 months at 25°C, (*E*) Sample *C* after 6 months at 60°C

Fig. 7. Visual changes in prepared tablets after heating at 30, 40, 50, and 60°C for 6 months (*left to right*)

component is consumed during the progression of the Maillard reaction with changes to other intermediate. These intermediates are all then changed to brown-colored melanoidines (8). Brown color development is almost similar in the presence and absence of magnesium-stearate-containing samples. It can be concluded that addition of magnesium stearate has not influenced the Maillard reaction progress significantly.

The HPLC chromatograms for intact brands were similar and the chromatogram for brand 3 is shown in Fig. 1d. The peak representing unknown-1 appeared at about 1 min.

According to Table I, in the absence of lactose (sample 9), baclofen was degraded up to 10% while in tablets in the dry state, the loss of drug was more than 20% (sample 13 and 15) except brand 1 (sample11). In wet conditions, the loss of drug content in brands is higher than the heated baclofen alone in wet conditions. In both the Maillard and caramelization reactions, highly UV-absorbing colorless compounds are formed at intermediate stages, whereas in the Maillard reaction, brown polymers are formed at the final stages (4,13,31). OD results showed a visible dark brown color development in wet conditions. Carbohydrates may undergo a caramelization reaction at very high temperatures. The brown color relates to brown non-nitrogenous water-soluble polymers named caramel colors (42,43). Results from samples 17–20 ruled out this type of reaction as no browning occurred.

CONCLUSION

According to results, the introduced HPLC method which uses a new internal standard is selective, linear, repeatable, accurate, and has intermediate precision. Thus, it can be used as a stability-indicating method.

Early-stage Maillard reaction product (ESMRP) which were characterized with tandem mass spectrometry showed that baclofen and lactose (either monohydrate or anhydrous) undergo the Maillard reaction. Condensation products of metoclopramide, amlodipine, and hydrochlorothiazide have been detected using mass spectrometry in a similar way by other scientists (7,18,25). It can be concluded that the most important factor affecting the Maillard reaction in prepared tablets and granules are physical contact and moisture content, respectively. FTIR analysis confirmed the formation of imine in the heated mixtures. Similar imine bonds have been reported in Maillard-type reactions but it has never been used in pharmaceutical evaluation of the Maillard reaction (37,38). Developing the brown color development is a key factor of the Maillard reaction occurrence and is also in accordance with the modern techniques used in this study.

HPLC analysis showed the ESMRP or unknown-1 peak in binary/tertiary mixtures of drug and lactose in the presence of magnesium stearate and also in solid-state formulations (granules and tablets) along with the brands tested. These findings indicate an incompatibility which takes place as a result of the Maillard reaction between a reducing sugar such as lactose and an amine-containing compound such as baclofen.

There are some reports of mutagenicity and carcinogenicity of Maillard reaction products on human cells in the nutritional literature (44), but the safety of the compounds that are generated during the Maillard reaction of baclofen and lactose remain to be investigated. It can be assumed that this type of reaction reduces drug potency and may cause some adverse effects relevant to drug safety. Therefore, it is advisable that lactose should be avoided in the formulation of baclofen.

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