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DSC, FT-IR, NIR, NIR-PCA and NIR-ANOVA for determination of chemical stability of diuretic drugs: impact of excipients

https://doi.org/10.1515/chem-2018-0014
received August 16, 2017; accepted December 23, 2017.

Abstract: It is well known that drugs can directly react with excipients. In addition, excipients can be a source of impurities that either directly react with drugs or catalyze their degradation. Thus, binary mixtures of three diuretics, torasemide, furosemide and amiloride with different excipients, *i.e.* citric acid anhydrous, povidone K25 (PVP), magnesium stearate (Mg stearate), lactose, D-mannitol, glycine, calcium hydrogen phosphate anhydrous ($CaHPO_4$) and starch, were examined to detect interactions. High temperature and humidity or UV/VIS irradiation were applied as stressing conditions. Differential scanning calorimetry (DSC), FT-IR and NIR were used to adequately collect information. In addition, chemometric assessments of NIR signals with principal component analysis (PCA) and ANOVA were applied.

Between the excipients examined, lactose and starch did not show any interactions while citric acid, PVP, Mg stearate and glycine were peculiarly operative. Some of these interactions were shown without any stress, while others were caused or accelerated by high temperature and humidity, and less by UV/VIS light. Based on these results, potential mechanisms for the observed interactions were proposed. Finally, we conclude that selection of appropriate excipients for torasemide, furosemide and amiloride is an important question to minimize their degradation processes, especially when new types of formulations are being manufactured.

Keywords: diuretics, excipients and interactions, high temperature and humidity, UV/VIS light, DSC, FT-IR, NIR, NIR-PCA, NIR-ANOVA

List of abbreviations:

$CaHPO_4$ - calcium hydrogen phosphate anhydrous
DSC - differential scanning calorimetry
Mg stearate - magnesium stearate
PVP - povidone K25
PCA - principal component analysis
PCs - principal components
SNV - standard normal variate

1 Introduction

Torasemide and furosemide belong to the group of loop diuretics which selectively inhibit NaCl reabsorption in the thick ascending limb of Henle´s loop. Amiloride belongs to potassium sparing diuretics which antagonize aldosterone effects at the late distal and cortical collecting tubules. This antagonism may be due to inhibition of sodium influx through ion channels in the luminal membrane [1]. Chemically, torasemide and furosemide are the sulfonamide derivatives while amiloride is a pyrazine compound with a guanidinium group. In the literature, only few papers exist in which chemical stability of torasemide and furosemide was examined in a solid state [2-6]. In addition, the experiments on the chemical stability of amiloride in a solid state have not been found in the literature. There

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is also scarce information about its interactions with excipients [7-10]. Furthermore, there are no reports about chemical stability of torasemide, furosemide and amiloride in which chemometric methods were added to typical analytical tools like differential scanning calorimetry (DSC) or spectrophotometry.

All guidelines emphasize the importance of testing chemical stability of drugs in final products, especially with respect to excipients. It is well known that drugs may directly react with excipients, for example in hydrolytic or oxidative way. In addition, excipients may have acidic or basic properties, depending on their chemical nature, and may contribute to the instability of pH labile drugs. Excipients may also affect drugs by altering the moisture content, which greatly increases the possibility of degradation. Moreover, excipients can be a new source of impurities that either directly react with drugs or catalyze their degradation [11].

Therefore, the purpose of the present study was to evaluate the impact of excipients on chemical stability of torasemide, furosemide and amiloride under standard laboratory conditions, at high temperature and humidity or under UV/VIS irradiation. To reach more definite conclusions, three analytical techniques, *i.e.* DSC, FT-IR and NIR as well as NIR together with chemometric assessments were applied.

2 Experimental Procedures

2.1 Drugs and excipients

Amiloride, furosemide, citric acid anhydrous, povidone K25 (PVP), magnesium stearate (Mg stearate), lactose, D-mannitol, glycine, calcium hydrogen phosphate anhydrous ($CaHPO_4$) and starch from Sigma Aldrich (USA), and torasemide from Biofarm Sp. z o.o. (Poland) were used. All materials were of pharmacopoeial purity and they were used as obtained. Each drug and each excipient were placed in tightly closed containers and then stored in a dessicator. Physical mixtures of the drugs with excipients were prepared at 1:1 ratio (*w/w*) by mixing the components in an agate mortar with a pestle for approximately 10 min. Then, the mixtures were placed in tightly closed containers and stored in a dessicator.

2.2 Stress conditions

Individual drugs, individual excipients and the mixtures were located in small alumina crucibles as thin layers *ca*. 3 mm. Then, these samples were stored in a climate chamber KBF P240 (WTC Binder, Germany) at high temperature and humidity (70°C/80% RH) for 30 and 60 days. The UV/VIS stress was performed in a photostability chamber Suntest CPS Plus (Atlas GmbH, Germany) equipped with a xenon lamp and a ID65 filter system. Two irradiance levels, *i.e.* 56706 kJ/m^2 and 113412 kJ/m^2, were applied while the temperature in the chamber did not exceed 35°C. After finishing each phase of the stress, the samples were transferred to tightly closed containers and stored in a dessicator until evaluation.

2.3 Analysis

A DSC Q200 calorimeter (TA Instruments, USA) was used to study thermal transformations of individual drugs, excipients and respective binary mixtures. The samples weighing 2-3 mg were closed in aluminum pans (Tzero Pan, TA Instruments), placed under a nitrogen pure atmosphere and heated from 0 to 370°C at a heating rate of 10°C/min. An empty aluminum pan was used as a reference.

FT-IR spectra were recorded on a Nicolet 6700 spectrometer (Thermo Scientific, USA), equipped with a Smart iTR accessory. After recording a background spectrum, the samples weighing 1-2 mg were placed on the diamond. Then, four scans were recorded for each sample over the range 4000-800 cm^{-1} with a resolution of 4 cm^{-1}.

Reflectance NIR spectra were measured using a Near IR Integrating Sphere from Thermo Scientific. Each spectrum was an average of four scans over the range 10000-4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4 Chemometrics

All chemometric computations were performed using a free GNU R computational environment, version 3.4.0. NIR spectra were standardized with Standard Normal Variate (SNV) algorithm to remove random shifts and random intensity changes, and to preserve only the shape information. Principal component analysis (PCA) was done without scaling because it is a standard strategy during spectral data analysis. ANOVA and MANOVA were used without further data pretreatment. The permutation tests were done in a standard configuration to see the distribution of ANOVA F coefficient among the permuted

Table 1: Differences between the stressed and the untreated binary mixtures of torasemide, furosemide and amiloride with operative excipients.

Method	Citric acid	PVP	Mg stearate	D-Mannitol	Glycine	$CaHPO_4$
Torasemide						
DSC	+	-	-	-	-	-
FT-IR	+	-	-	-	-	-
NIR	+	-	-	-	-	-
NIR-ANOVA	-	-	+	-	-	-
Furosemide						
DSC	+	+	-	-	+	-
FT-IR	+	+	+	-	++	-
NIR	+	-	+	-	+	-
NIR-ANOVA	+	-	+	+	-	+
Amiloride						
DSC	+	+	-	-	+	-
FT-IR	+	+	-	-	-	-
NIR	-	-	-	-	+	-
NIR-ANOVA	-	+	-	-	+	+

+ the temperature/humidity influence was observed; ++ the temperature/humidity and UV/VIS light influences were observed

datasets. PCA, ANOVA and MANOVA were carried out by the built-in functions "princomp", "aov" and "manova", respectively while permutation tests were conducted using "lmPerm" extension package with a function "aovp".

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 General

The binary mixtures were prepared in 1:1 proportions of each drug to each excipient in order to maximize interactions. First, the individual components and their mixtures were analyzed using DSC, FT-IR and NIR. Secondly, the influence of stress on the individual components and their binary mixtures was evaluated using the same methods. In the next step, the differences between the stressed mixtures and the untreated ones were studied. At this stage, chemometric assessments of the NIR spectra were added. Only the differences which appeared at both levels of stress, *i.e.* after storing under 70°C/80% RH for 30 and 60 days or after irradiation with 56706 kJ/m^2 and 113412 kJ/m^2, were taken into account. Occurrence of these differences assigned to the applied methods are summarized in Table 1. However, only the differences which were confirmed by at least two applied methods were taken into account in our further discussion.

3.2 DSC experiments

The details of thermal processes observed for individual drugs and excipients (not stressed) are listed in Table 2. Generally the presented results are, both qualitatively and quantitatively, similar to the results published by other authors [6,9,12-16].

High temperature and humidity as well as UV/VIS light did not influence the thermal processes of torasemide. Similarly, high temperature and humidity did not affect any thermal process of furosemide. However, UV/VIS irradiation of individual furosemide caused shifting the exotherm from 220°C to 210°C and the second endotherm from 270°C to 280°C. In the case of amiloride, the stress caused shifting the first endotherm to 115°C (high temperature and humidity) or to 123°C (UV/VIS irradiation). Thermal processes of melting and decomposition of citric acid were influenced by high temperature and humidity. It was observed as moving the first endotherm from 55°C to 112°C and broadening the third one at 214°C. For glycine and Mg stearate, slight broadening of the melting endotherms was observed for the stressed samples (high temperature and humidity). In the case of PVP, the influence of high temperature and humidity was observed as flattening the first endotherm at 81°C and shifting the second one from 185°C to 152°C.

As far as binary mixtures were concerned, the changes were observed for torasemide with citric acid, furosemide with citric acid, PVP and glycine, and amiloride with citric acid, PVP and glycine, even if the mixtures were not stressed.

Table 2: DSC and FT-IR characteristics of torasemide, furosemide, amiloride and operative excipients (not stressed)

Drug or excipient	DSC		FT-IR	
	Tm [C°]	Enthalpy [kJ/mol]	Bands [cm^{-1}]	Vibrations
Torasemide	165(endo) melting	36.8	3349	N-H stretching
			2977	C-H stretching
			1693	C=O stretching
			1608, 1396, 1262, 1233	
			1109	SO_2 stretching
			1045	C-N stretching
Furosemide	137 (endo) melting	2.65	3398, 3351, 3282	N-H (Ar-NH-CH_2)
	220 (exo)	42.3	1667	C=O stretching
	270 (endo)	11.6	1590, 1561	SO_2NH_2
			1262, 1238	C-O stretching
			1140	SO_2 stretching
Amiloride	130 (endo)	80.8	3294, 3165	N-H stretching
	210 (exo)	2.3	1543	N-H bending
	290 (endo) melting	29.3	1675	C=O stretching
			1246	N-aromatic ring
Citric acid	55 (endo)	36.6	3491	O-H (COOH)
	155 (endo) melting	38.4	1752	C=O (COOH)
	214 (endo)	122.8	1719	
			1207	C-OH stretching
			1169	C-OH bending
PVP	81 (endo)	244.6	3395	O-H stretching
	185 (endo)	16.1	2951	C-H stretching
			1646	C=O stretching
			1424	
			1287	C-N stretching
			1166	
Mg stearate	92 (endo)		2954, 2917, 2848	C-H stretching
	116 (endo) melting	134.1(cumulatively)	1577	COO^- stretching
	121 (endo)		1337	
Glycine	254 (endo)	74.1	3152	NH_3^+ stretching
			3008	
			2824, 2703, 2602, 2517	NH_3^+ and C-N stretching
			1577	COO^- stretching
			1502	NH_3^+ bending
			1178, 1032	C-N stretching

The thermogram of torasemide mixed with citric acid was considerably different from the thermograms of both individual components. A new endotherm at 130°C appeared and all thermal processes above 150°C fused into one broad endotherm with maximum around 180°C. Such broadening indicates interactions, more specifically hydrogen bonds between the N-H group of the drug and the C=O group of citric acid. After the stress, both endotherms overlapped and the main body of these thermal processes shifted to higher temperatures (Figure 1a). However, the enthalpy values for these endotherms were similar in the untreated and in the stressed mixtures (71.8-69.4 kJ/mol).

In the mixture of furosemide with citric acid, the exotherm at 220°C due to decomposition of furosemide disappeared and a wide endotherm between 180°C and 220°C with enthalpy equal 60.3 kJ/mol appeared, suggesting that decomposition of the drug was intensified in the presence of citric acid. In the stressed mixture, this endotherm slightly enlarged (to 64.4 kJ/mol) and shifted to around 200°C. At the same time, the peak of furosemide melting enlarged and shifted from 137°C to 144°C, probably as a result of overlapping with the melting endotherm of citric acid which shifted from 155°C to lower temperatures. The enthalpy value of this overlapped process was 17.9 kJ/mol. When the untreated mixture of furosemide with citric acid was compared, the enthalpy value of furosemide melting was 3.9 kJ/mol and the enthalpy value of citric acid melting was 43.8 kJ/mol (Figure 2a).

In the mixture of furosemide with PVP, main thermal processes of both components were observed without considerable temperature shifting while the enthalpy

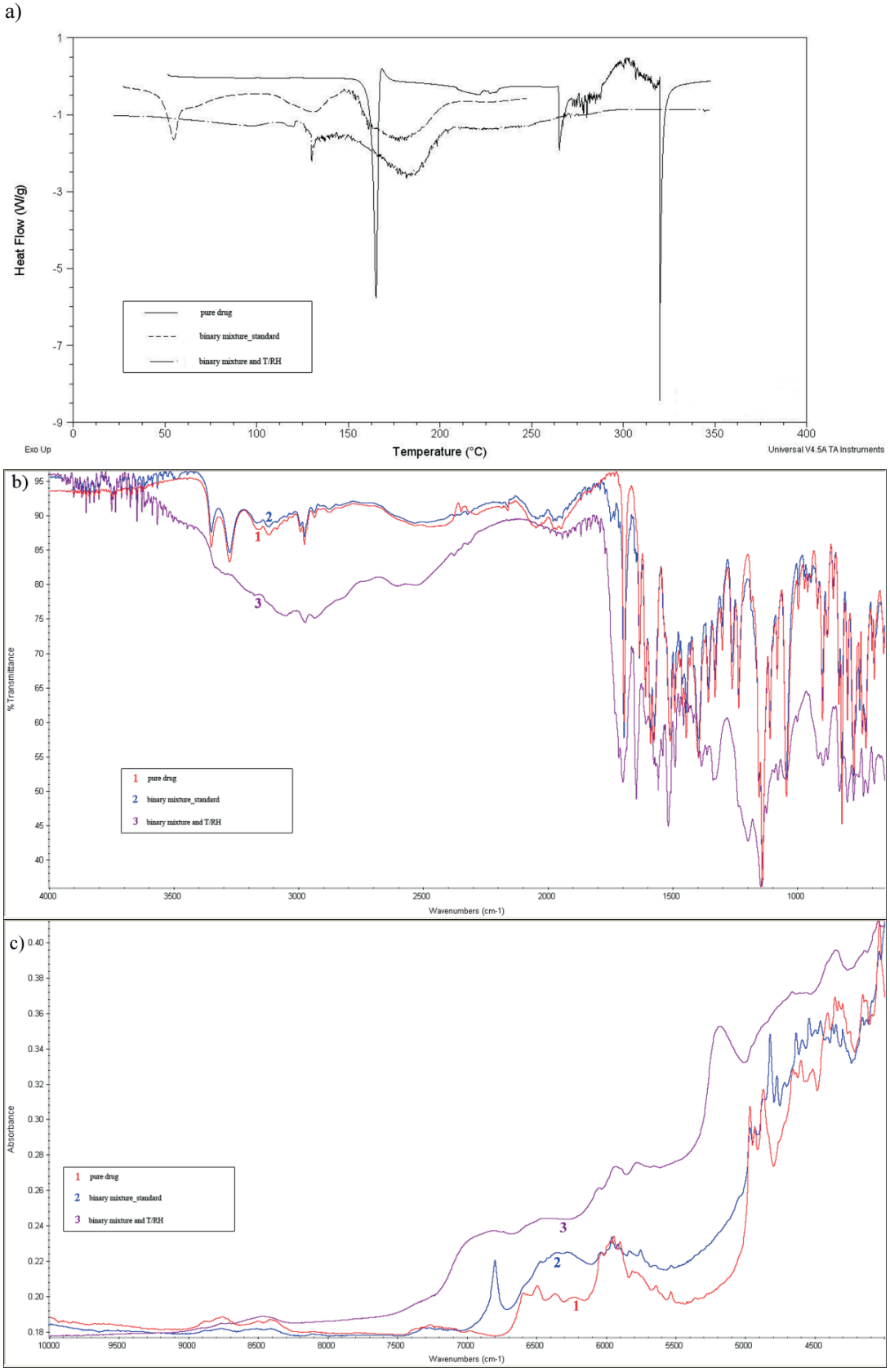

Figure 1: Torasemide and citric acid: DSC scans (a), FT-IR spectra (b) and NIR spectra (c) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure torasemide.

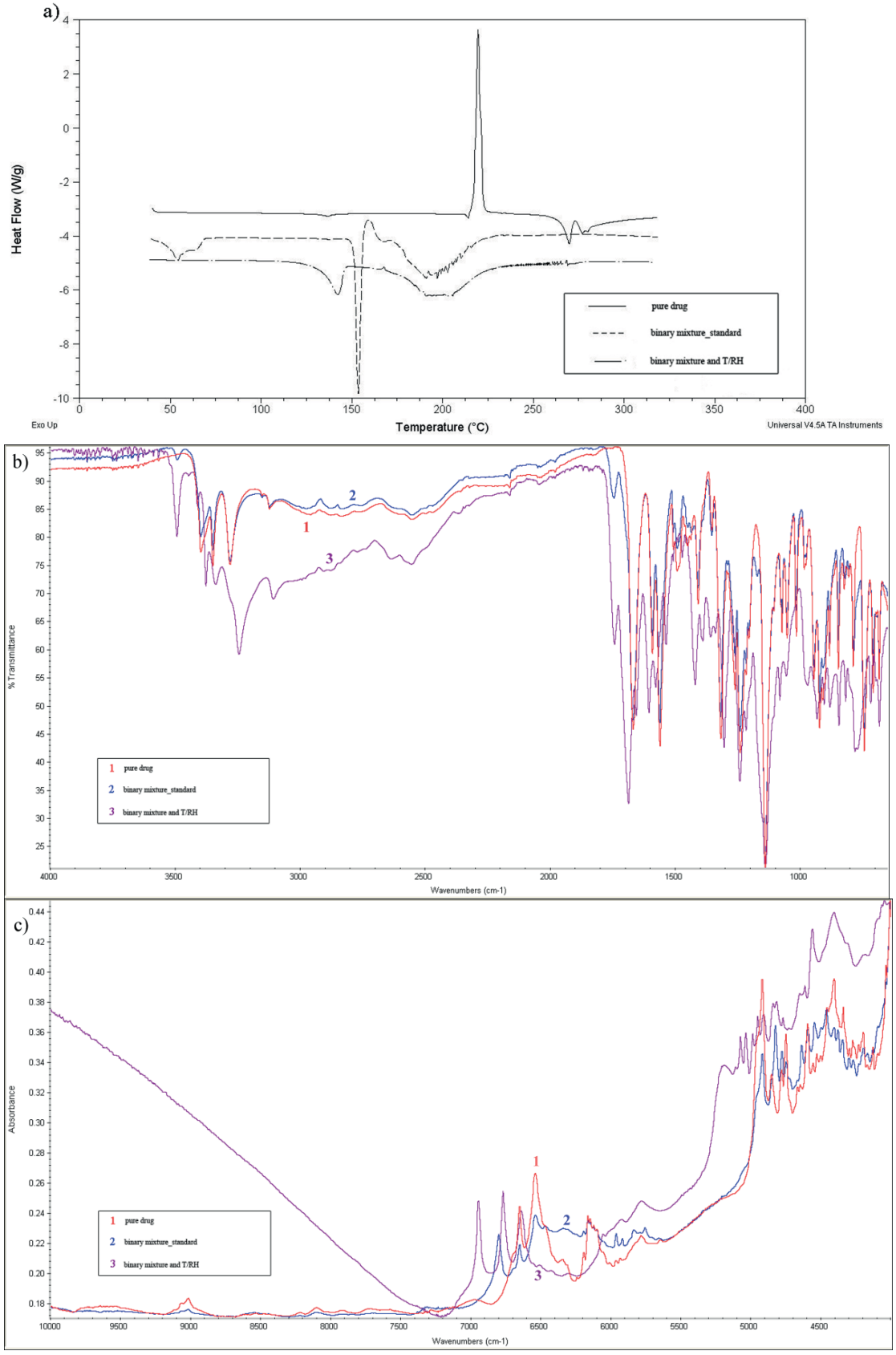

Figure 2: Furosemide and citric acid: DSC scans (a), FT-IR spectra (b) and NIR spectra (c) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure furosemide.

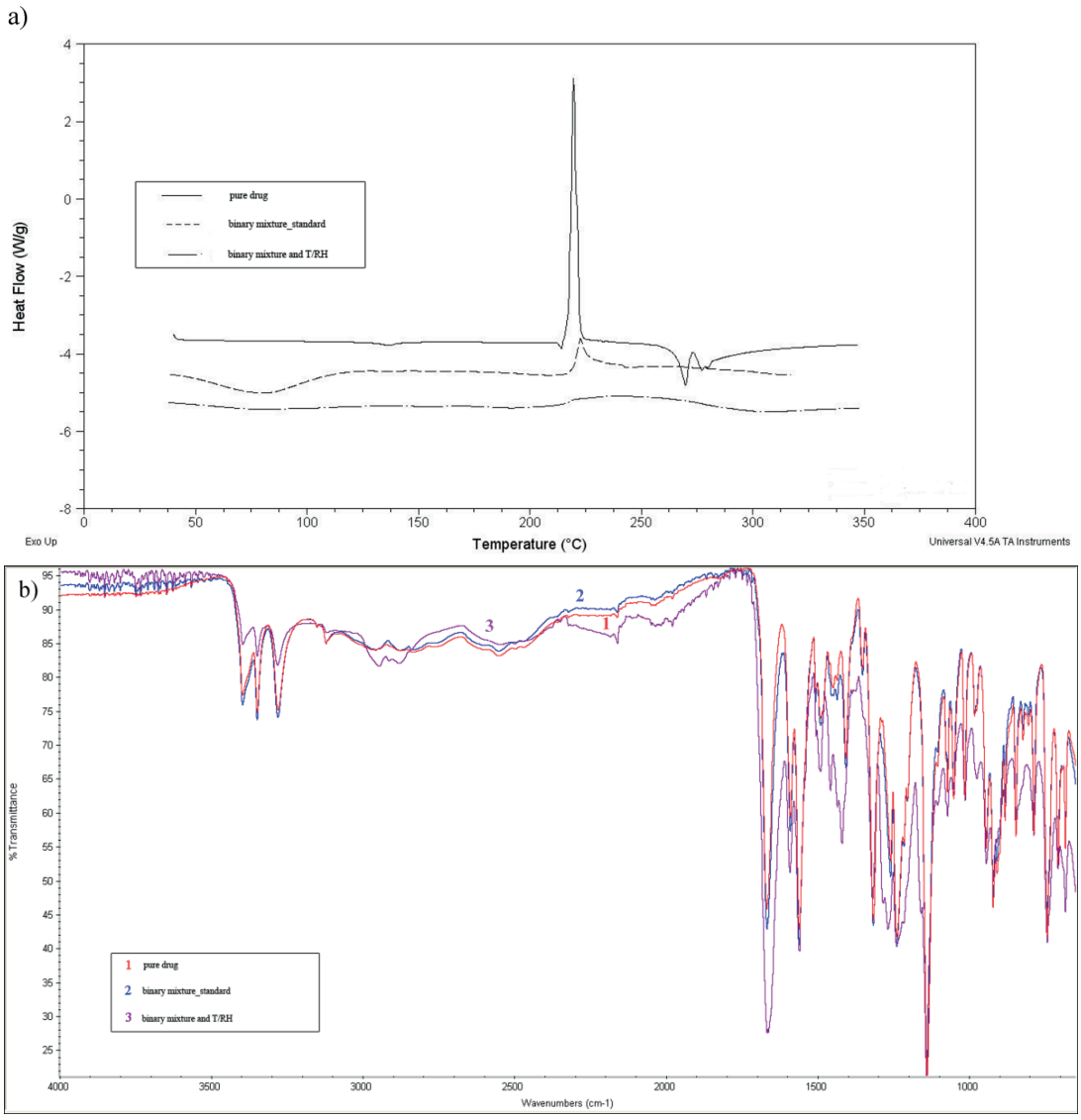

Figure 3: Furosemide and PVP: DSC scans (a) and FT-IR spectra (b) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure furosemide.

of furosemide exotherm at 220°C increased to 45.6 kJ/mol in comparison to 42.3 kJ/mol for the individual drug. In addition, high temperature and humidity stress resulted in an essential blurring of all thermal processes (Figure 3a).

In the mixture of furosemide with glycine, the exotherm at 220°C and the endotherm at 270°C, both of furosemide, and the endotherm at 254°C of glycine were replaced by a broad endotherm between 210°C and 250°C. Because the peaks corresponding to the drug and the excipient were shortened and broadened, formation of hydrogen bonds between furosemide and glycine was probable. For the stressed mixture, the fused broad endotherm was narrower with lower enthalpy value, *i.e.* 59.8 kJ/mol versus 72.3 kJ/mol for the untreated mixture. Therefore, we expected that degradation of furosemide was facilitated in the presence of glycine. Another effect of the stress was observed as a decrease of the endotherm due to melting of furosemide at 137°C (Figure 4a).

In the mixture of amiloride with citric acid, the melting endotherms of both components shifted to lower temperatures and probably fused. The observed shifting indicates formation of intermolecular hydrogen bonds between the N-H group of amiloride and the C=O group of citric acid. In the untreated mixture, three distinct endotherms between 100°C and 200°C were observed with total enthalpy equal 78.3 kJ/mol. After stressing,

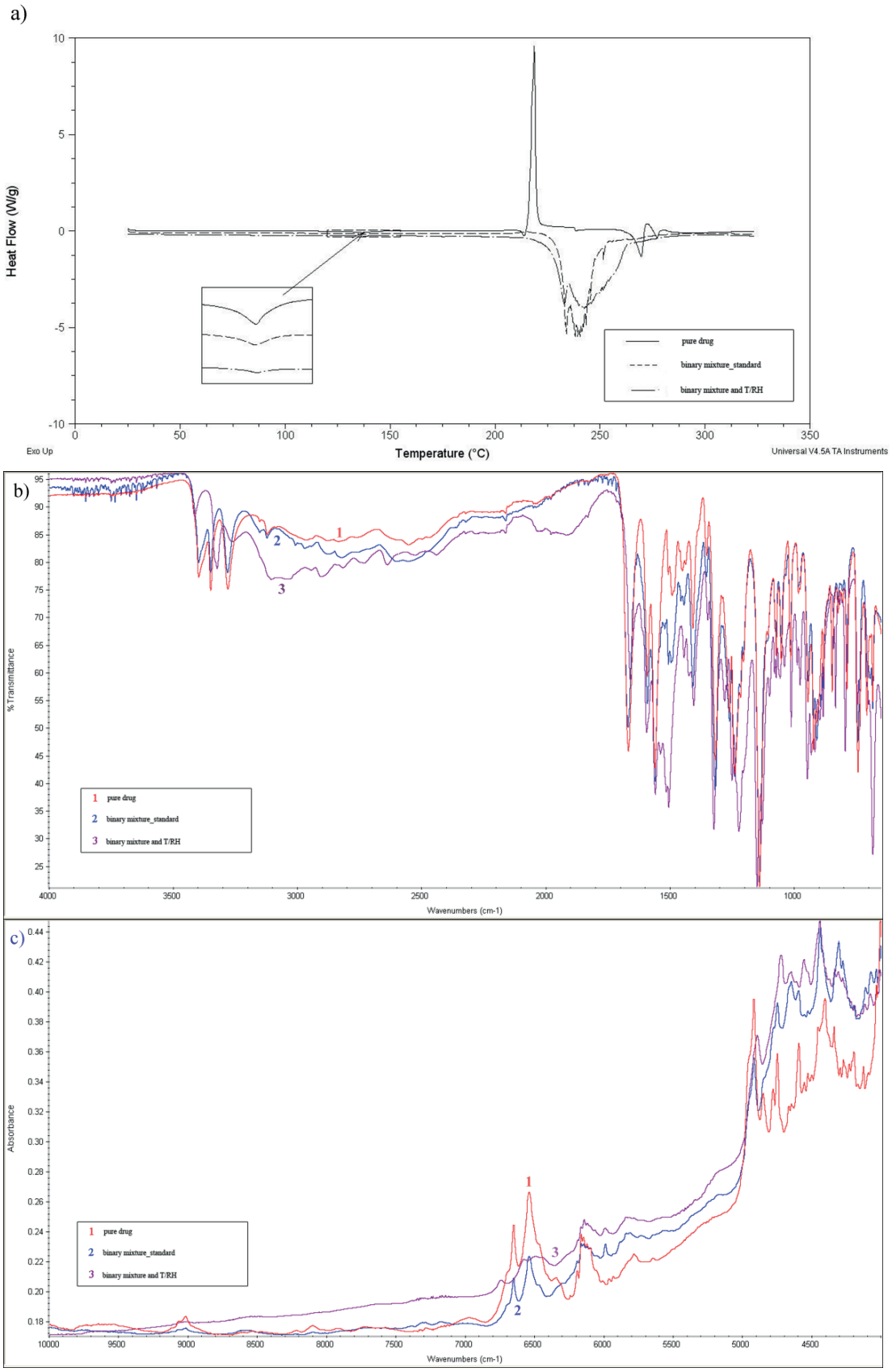

Figure 4: Furosemide and glycine: DSC scans (a), FT-IR spectra (b) and NIR spectra (c) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure furosemide.

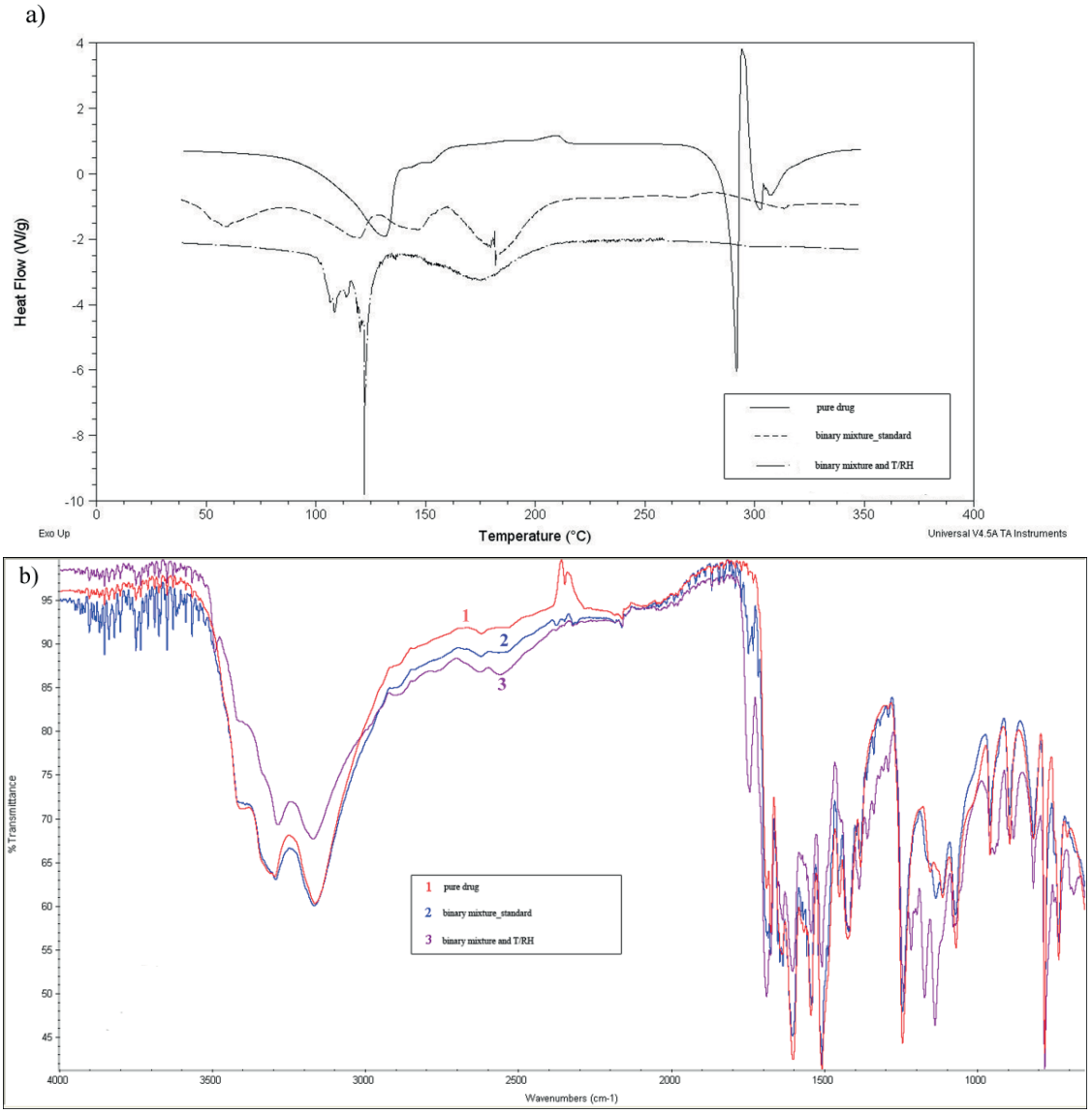

Figure 5: Amiloride and citric acid: DSC scans (a) and FT-IR spectra (b) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure amiloride.

total enthalpy for these peaks decreased to 72.3 kJ/mol (Figure 5a).

In the mixture of amiloride with PVP, the main thermal processes of both components were not affected. However, high temperature and humidity stress resulted in an essential blurring of all thermal processes (Figure 6a).

In the mixture of amiloride with glycine, a strong effect of glycine itself on all thermal processes of amiloride was observed. The main endotherm of amiloride decreased from 80.8 kJ/mol for an individual drug to 26.0 kJ/mol in the mixture. At the same time, its second endotherm almost disappeared, probably because of fusion with the endotherm of glycine which shifted to 245°C. This observation suggests formation of hydrogen bonds between the N-H group of amiloride and the C=O group of glycine. After high temperature and humidity stress, the first endotherm of amiloride broadened and flattened to 4.9 kJ/mol. In addition, the enthalpy of the main thermal process rose to 75.5 kJ/mol in the stressed mixture (from 40.9 kJ/mol in the untreated mixture) (Figure 7a).

The DSC scans obtained for the mixtures of torasemide, furosemide and amiloride with lactose, D-mannitol, $CaHPO_4$ and starch did not show significant changes, for both, the untreated mixtures and the stressed ones.

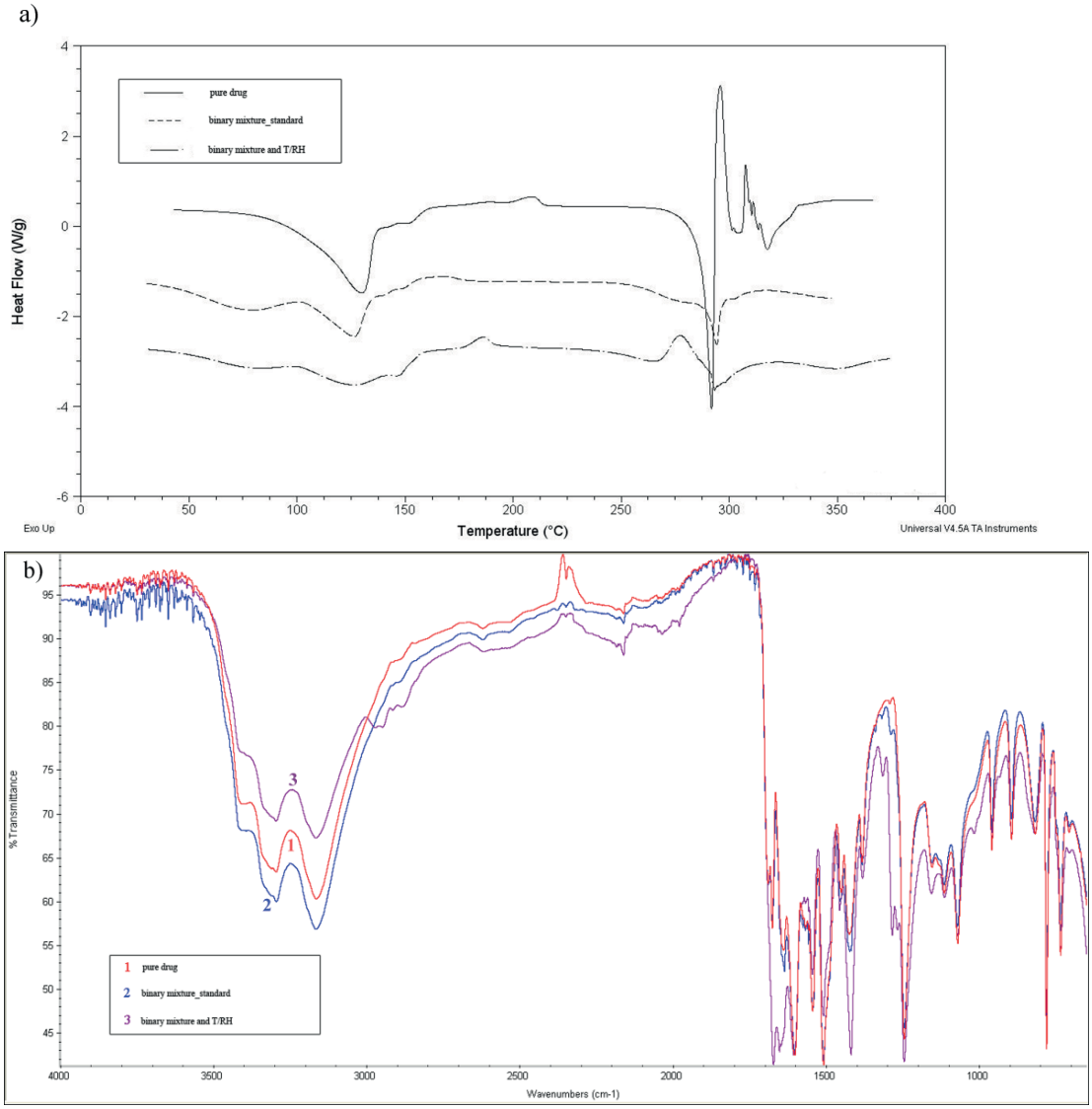

Figure 6: Amiloride and PVP: DSC scans (a) and FT-IR spectra (b) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure amiloride.

3.3 FT-IR study

The characteristics of the spectra for individual drugs and individual excipients (non stressed) are presented in Table 2. Generally, the presented results are similar to the results published by other authors [6,7,14,15].

From the spectra obtained for the individual drugs stressed at high temperature and humidity or under UV/VIS light, it was clearly seen that torasemide, furosemide and amiloride did not change. Also, the individual excipients did not show changes after the stress. On the contrary, FT-IR spectra of the binary mixtures, even if the mixtures were not stressed, showed changes for torasemide with citric acid, furosemide with citric acid, PVP, Mg stearate and glycine, and amiloride with citric acid and PVP. In addition, the mixture of furosemide with glycine was affected by UV/VIS light.

The spectrum of the untreated mixture of torasemide with citric acid showed flattening of a broad peak at 3349 cm^{-1} due to N-H vibrations of torasemide. At the same time, the peak at 1719 cm^{-1} due to C=O vibrations of citric acid shifted to 1690 cm^{-1} and overlapped that of torasemide. The observed shifting to lower wavenumbers suggests formation of intermolecular hydrogen bonds between the N-H group of torasemide and the C=O group of citric acid which was also shown by our DSC results. When this mixture was treated with high temperature and humidity, new changes in the spectrum were observed as shifting the

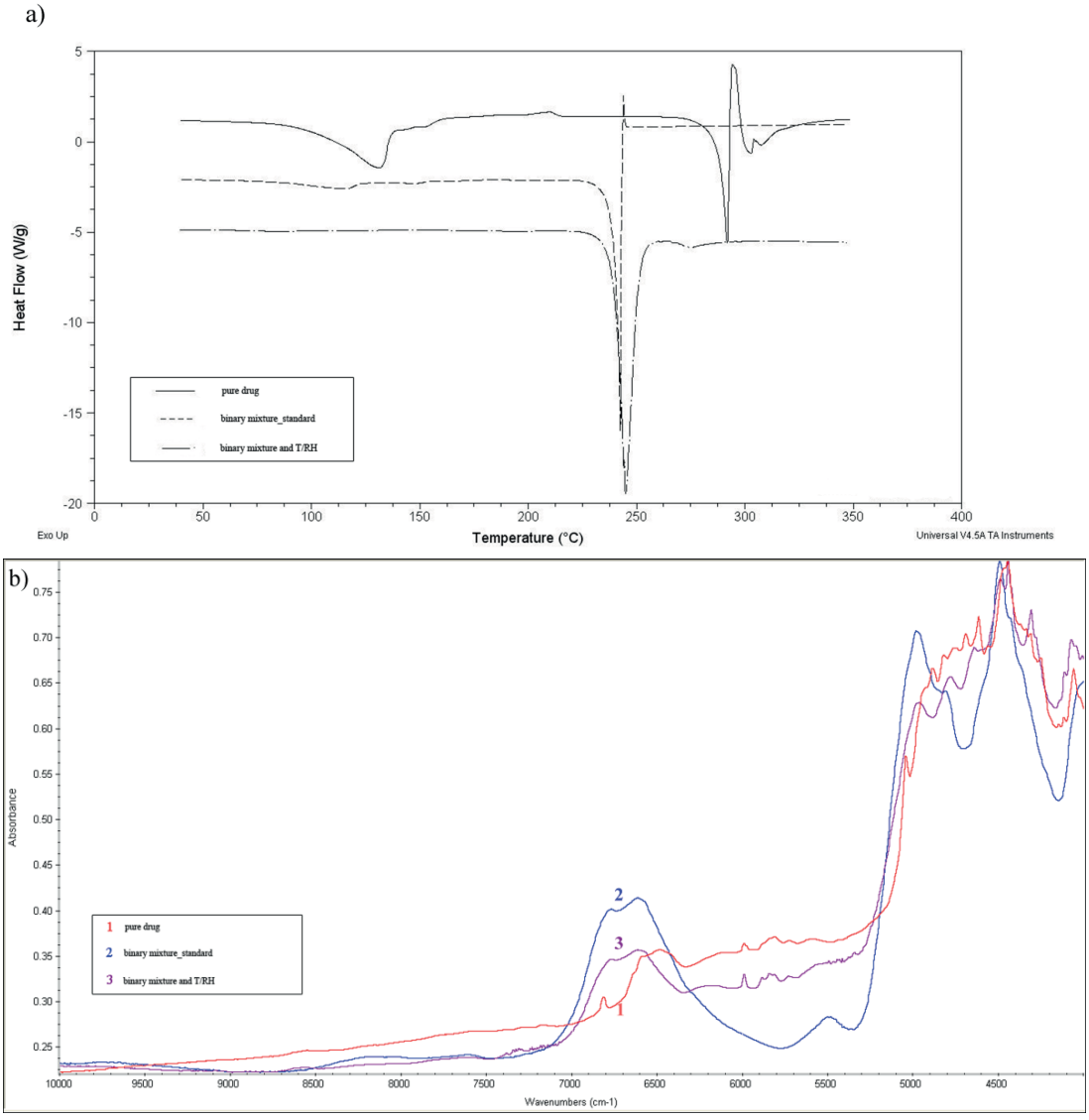

Figure 7: Amiloride and glycine: DSC scans (a) and NIR spectra (b) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure amiloride.

characteristic peaks of torasemide from 1109 cm^{-1} and 1045 cm^{-1} (Figure 1b). It is known that a humid environment, high temperature and acidic additives can facilitate hydrolytic reactions [17]. Therefore the acid catalyzed hydrolysis of the sulfonyl urea moiety of torasemide was possible in our conditions (Figure 8a). Similarly, in the study of Jovic et al. [2] and Patel et al. [3], the acid catalyzed hydrolysis of torasemide through the loss of an amine from the sulfonyl urea, yielding the sulfonamide group, was confirmed. In addition, data from the literature show that citric acid may facilitate degradation of some drugs in a solid state, *e.g.* quinapril [18] and atorvastatin [19]. In the mixture of furosemide with citric acid, even if the mixture was not stressed, the characteristic peaks of citric acid at 3228 and 3350 cm^{-1} almost disappeared. We expected that in the presence of furosemide, the intramolecular hydrogen bonds in the citric acid molecule were weakened. When this mixture was treated with high temperature and humidity, the changes were observed as appearance of new bands in the region 3100-3550 cm^{-1} attributed to the amine group (Figure 2b). It is known that furosemide is a labile substance and its main degradation product is 4-chloro-5-sulfamoyl anthranilic acid [6]. Based on the observed changes we suppose that citric acid may intensify such degradation of the drug (Figure 8b).

In the literature, solid dispersions of furosemide with PVP were frequently studied [7-9,20]. Bearing in mind the structures of both components, hydrogen bonding

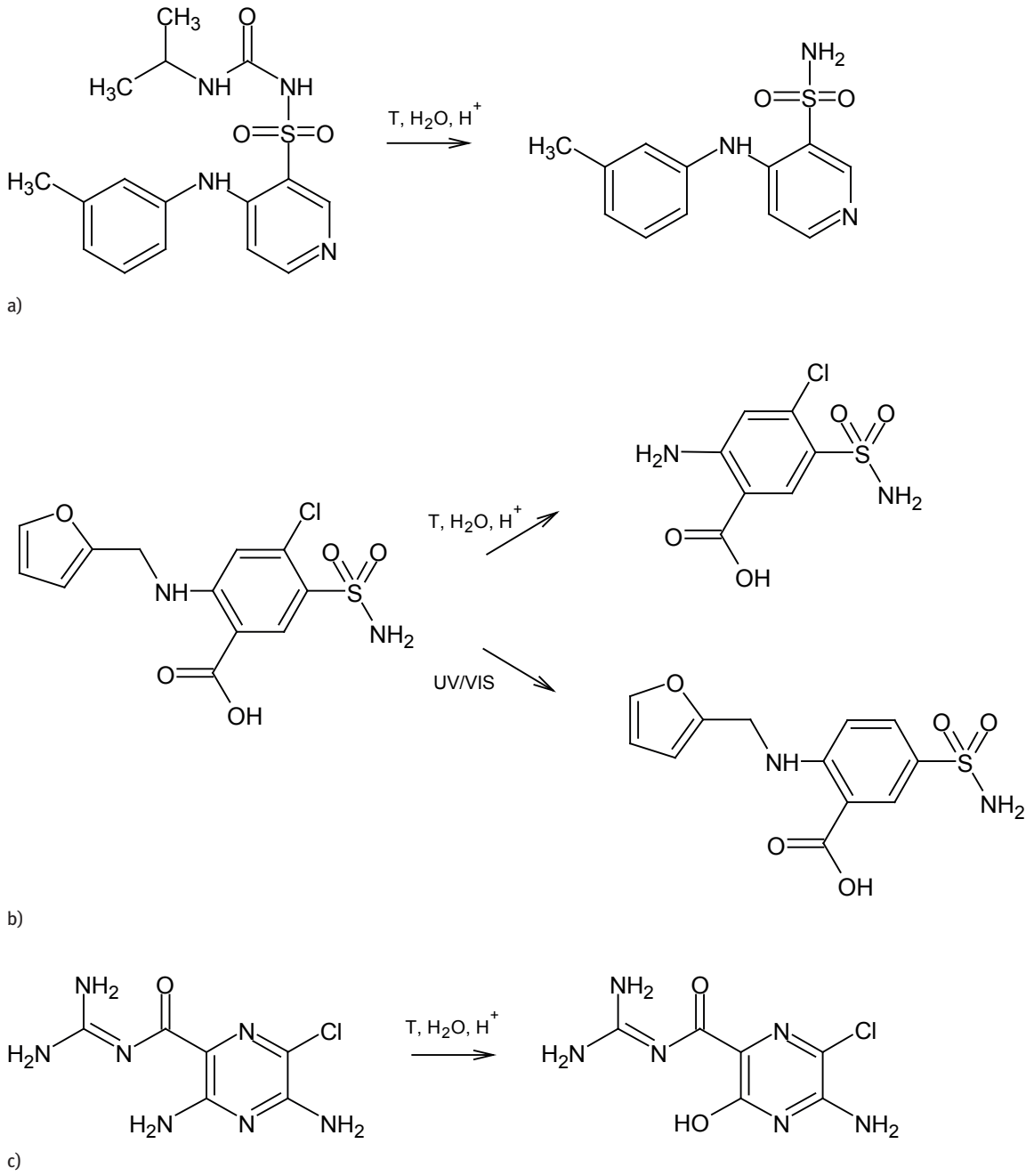

Figure 8: Hypothetical degradation pathways of torasemide (a), furosemide (b, c) and amiloride (d) influenced by operative excipients or stress conditions.

between the O-H group of the drug and the C=O group of the excipient can be expected. However, in our experiment, the FT-IR spectrum of this mixture did not show formation of hydrogen bonds between the components. Similarly, hydrogen bonding between furosemide and PVP was not observed using DSC, FT-IR and powder X-ray diffraction in the study of Patel et al. [9]. On the other hand, Kaminska et al. [20] supposed that hydrogen bonding between furosemide and PVP catalyzes chemical degradation of furosemide to 4-chloro-5-sulfamoyl anthranilic acid. This fact is quite intriguing but our FT-IR experiment does not confirm this. Moreover, even at high temperature and humidity, we only observed that the peaks of furosemide at 1262 and 1238 cm^{-1} gently changed their shapes while the peak of PVP at 1166 cm^{-1} slightly decreased (Figure 3b).

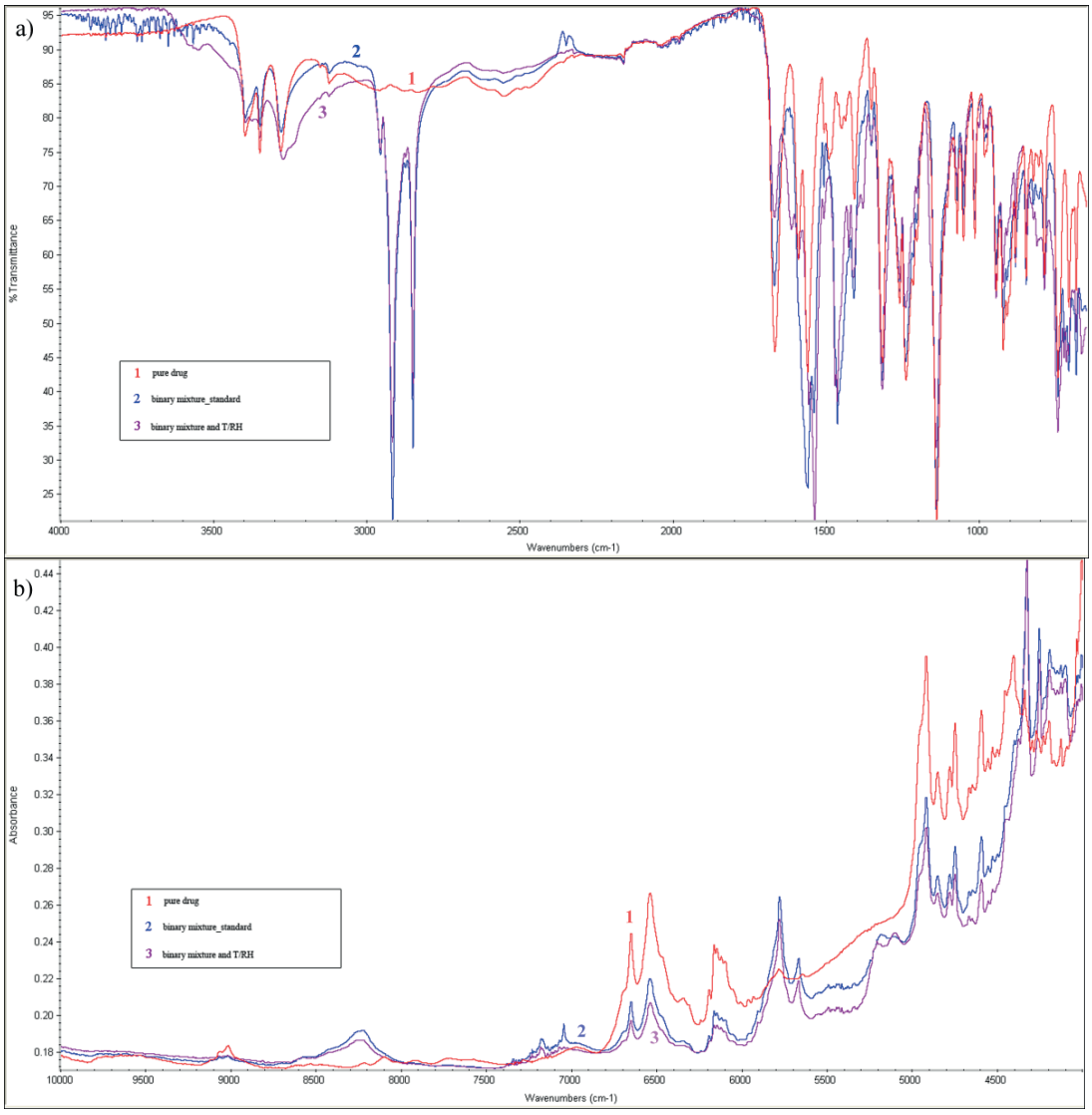

Figure 9: Furosemide and Mg stearate: FT-IR spectra (a) and NIR spectra (b) for the mixture stressed with high temperature/humidity (T/RH) and the untreated mixture (standard) against pure furosemide.

The spectrum obtained for the mixture of furosemide with Mg stearate showed the lack of the peak at 1577 cm^{-1} due to COO^- group of Mg stearate. Therefore, the interaction via hydrogen bonding of furosemide with the COO^- group of Mg stearate can be taken into account. In the literature, similar interactions between captopril and Mg stearate was confirmed [21]. Another important factor to consider is alkalinity of Mg stearate. When this lubricant is present in solid mixtures, it may increase the pH of the microenvironment and in consequence, accelerate alkaline hydrolysis. When our mixture of furosemide with Mg stearate was treated with high temperature and humidity, the lack of the peak at 3398 cm^{-1} and broadening the peak at 3282 cm^{-1}, both due to the secondary amine group of the drug, were observed (Figure 9a). Based on these changes we suppose that the amine group of furosemide may be affected in presence of Mg stearate that was previously reported for drugs containing amine groups [22].

The spectrum of furosemide mixed with glycine, even if the mixture was not stressed, showed the lack of peaks corresponding to NH_3^+ and COO^- groups of glycine. This disappearance suggests solid state interactions between the components. Taking together our DSC and FT-IR results, the possibility of hydrogen bonding via the COO^- and NH_3^+ groups of glycine could be taken into account, however we did not find information about similar interactions. When this mixture was treated with high temperature and humidity, the changes were observed as the lack of peaks at 3398, 3351 and 3282 cm^{-1} due to Ar-NH-CH_2 group of furosemide (Figure 4b). Therefore, we suppose that

glycine facilitates degradation of furosemide to 4-chloro-5-sulfamoyl anthranilic acid (Figure 8b).

The mixture of amiloride with citric acid showed the changes, even if the mixture was not stressed. The peak of citric acid at 1752 cm^{-1} shifted to 1690 cm^{-1} confirming some changes in its C=O group. The amiloride molecule comprises a guanidine group and several amino groups which have very good functionality for hydrogen bonding. The observed shifting to a lower wavenumber suggests formation of intermolecular hydrogen bonds between the C=O group of citric acid and the N-H group of amiloride, similarly to our DSC results. When high temperature and humidity were applied to this mixture, a new band at 3520 cm^{-1} appeared. In the literature, the possibility of acid catalyzed hydrolysis of amiloride with converting one of the NH_2 group on the pyrazine ring to the OH group was described (Figure 8c) [23]. Another possibility is forming the amide group in the presence of citric acid. In the literature, such interaction was described for the mixture of citric acid with carvedilol [24]. In our experiment, however, the peak due to the C=O amide group of amiloride was not clearly seen (Figure 5b).

The mixture of amiloride with PVP showed the spectrum with some peaks shifted around 2-3 cm^{-1} and with small reduction of the intensity of these peaks. At the same time, the peak of PVP due to the C=O group was not affected suggesting the lack of hydrogen bonds, although in the literature, the possibility of hydrogen bonding between the C=O group of PVP and the NH_2 group of amiloride was shown [22,25]. After high temperature and humidity stress, we observed that the peak corresponding to the C=O group broadened and shifted to a lower wavenumber but we could not draw a credible conclusion from this observation (Figure 6b).

In the mixtures of torasemide, furosemide and amiloride with lactose, D-mannitol, $CaHPO_4$ and starch, the changes in the FT-IR spectra were not significant, for both the untreated mixtures and the stressed ones.

3.4 NIR experiments

From the obtained NIR spectra it was seen that torasemide, furosemide and amiloride themselves did not visibly change at high temperature and humidity or under UV/VIS irradiation. Also, the NIR spectra of individual excipients did not show changes after the stress. Similarly, the NIR spectra obtained for the mixtures of torasemide, furosemide and amiloride with lactose, D-mannitol, $CaHPO_4$ and starch, did not show changes in the nature of the absorption patterns, for both the untreated mixtures and the stressed ones. However, the rest of the excipients showed interactions, even if these mixtures were not stressed. It was clearly seen for torasemide with citric acid (Figure 1c) and furosemide with citric acid (Figure 2c). In addition, the spectra of these two mixtures showed visible changes at high temperature and humidity. Additionally, interactions of torasemide and furosemide with citric acid were also shown by our DSC and FT-IR results. Next, the NIR spectra showed interactions for the stressed mixtures of furosemide with Mg stearate (Figure 9b), furosemide with glycine (Figure 4c) and amiloride with glycine (Figure 7b). As far as the mixture of furosemide with glycine was concerned, the interactions were also shown by our DSC and FT-IR results. On the other hand, the NIR spectra did not show changes after UV/VIS irradiation.

It is known that direct interpretation the NIR profiles is difficult. Moreover, the discussion on the visually observed differences in the NIR region can be subjective to some extent. Therefore we decided to use chemometric methods to objectively evaluate the differences between the NIR spectra of the stressed and the untreated mixtures.

Before chemometric treatment, respective NIR spectra were scaled to unit variance and zero mean (SNV algorithm). This standard procedure reduced random spectral shifts and preserved that only the shape of the spectra was taken into account. Then, the spectra were subjected to PCA in order to reduce the dimensionality. As a result, the first five principal components (PCs) accounted for 36.9%, 19.0%, 16.2%, 12.4% and 6.1% of the explained variance, respectively.

Inspecting the first loading value (Figure 10a), it was concluded that the main source of variability was connected with an increase of absorbance in the region 4500-5000 cm^{-1} (the combinations of N-H and O-H) and the region 5800-7300 cm^{-1} (the first overtones of C-H, N-H and O-H). The second trend (Figure 10b) was related to an increase of absorbance in the region 4400-5100 cm^{-1} (the C-H and O-H combinations) and the region 6200-6350 cm^{-1} (the first overtones of N-H). However, there was a difference within the region 5000-6200 cm^{-1}, where the loading value was negative. In this region, the combinations of O-H, the first overtones of C-H and the second overtones of C=O occurred. The third PC (Figure 10c) represented a trend where the region 4600-4800 cm^{-1} (the combinations of O-H and N-H) and the region 5500-6700 cm^{-1} (the first overtones of C-H and the second overtones of C=O) had their absorbance increased, together with a decrease in the region 5500-4800 cm^{-1} (the combinations of O-H). The fourth and fifth PCs were rather noisy so we did not

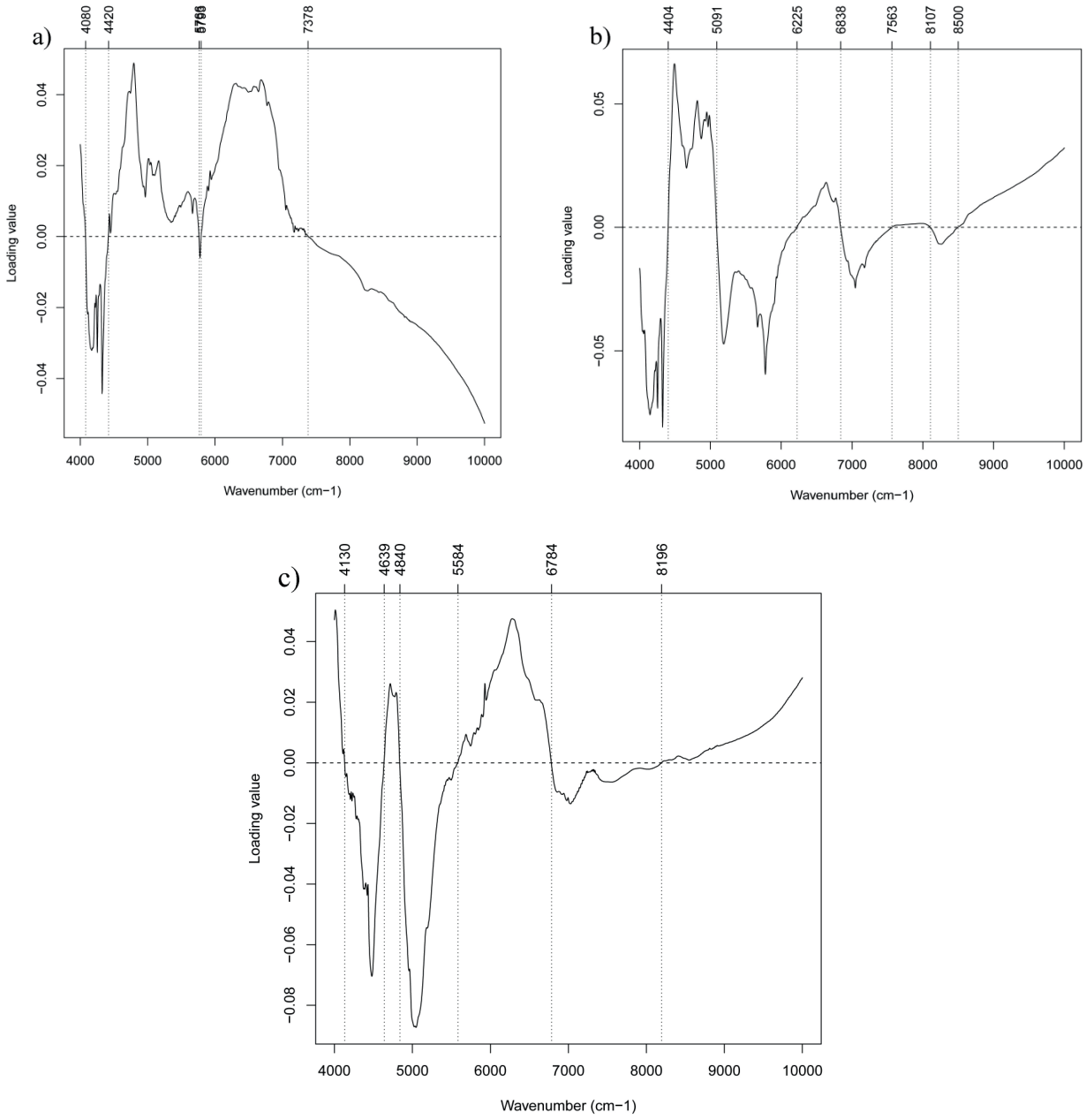

Figure 10: Loading values of the first (a), the second (b) and the third (c) PCs of the NIR dataset.

interpret them. Summarizing the first, second and third PC observations we conclude that these trends confirm our FT-IR and DSC results because the N-H and C=O groups are the main source of variation.

In the next step, the PCs were used as a new dataset in multi-way analysis of variance. The preliminary experiments with built-in univariate multi-way ANOVA (with the first PC only) and multivariate multi-way ANOVA (with several first PCs inserted into the model) gave similar results with increasing significance (decreasing p value) when subsequent components were included. This is a normal phenomenon in such analysis and in the present experiment, taking one from several PCs was the best choice.

Being aware about deviations from normality what raises a question about reliability of p values, we decided to perform the univariate multiway ANOVA based on the permutation tests. The dataset was split into three subsets for each drug. In each subset, two-way interactions were checked between the presence of excipients and various stress conditions (the second factor with several levels).

The results of the permutation ANOVA showed a significant influence of excipients on the degradation processes for the following mixtures: torasemide with Mg stearate ($p<1\times10^{-5}$), furosemide with citric acid ($p<1\times10^{-5}$), furosemide with Mg stearate ($p=0.0102$), furosemide with D-mannitol ($p=0.03919$), furosemide with $CaHPO_4$ ($p=0.0022$), amiloride with PVP ($p<1\times10^{-5}$), amiloride with Mg stearate ($p<1\times10^{-5}$), amiloride with glycine ($p<1\times10^{-5}$) and amiloride with $CaHPO_4$ ($p<1\times10^{-5}$). These results confirmed the interactions of furosemide with citric acid, furosemide with Mg stearate, amiloride with PVP as well as amiloride and glycine. Thus, our results obtained by NIR-ANOVA were in partial agreement with the results obtained by DSC, FT-IR and NIR methods (Table 1), although some differences were not surprising. It is obvious that the DSC results contain additional information about the heating of the samples, whereas IR signals are registered at room temperature. Moreover, the NIR spectra contain different information than the FT-IR ones.

3.5 Photoreactivity of torasemide, furosemide and amiloride

Torasemide shows significant absorption above 300 nm suggesting its potential photoreactivity. However, torasemide in a solid state was stable under photolytic conditions equal 1.2 million luxh and 200 Wh/m^2. Also, there was no significant degradation when the drug was exposed to similar light energy in solution [2]. In our study, torasemide did not show degradation in a solid state under 56706 kJ/m^2 and 113412 kJ/m^2 irradiation which constituted three and six times bigger doses of energy. Therefore, photostability of torasemide was confirmed.

It is well known that furosemide in solution undergoes photochemical degradation through hydrolysis and oxidation to form two products, 4-chloro-5-sulfamoylanthranilic acid and *N*-furfuryl-5-sulfamoyl anthranilic acid [6,7]. However, in a solid state, the drug was less sensitive to the light resulting in *ca*. 1.5% degradation after exposition to 2.1×10^6 luxh and 576 Wh/m^2 [4,5]. In the present experiment, the DSC scan of furosemide stressed by UV/VIS light showed shifting the exotherm from 220°C to 210°C and the second endotherm from 270°C to 280°C suggesting that furosemide components responsible for this process changed. In addition, the FT-IR spectrum of the stressed mixture of furosemide with glycine showed the lack of peaks at 3398, 3351 and 3282 cm^{-1}due to the Ar-NH-CH_2 group of the drug. Therefore, we conclude that furosemide in a solid state may be degraded by UV/VIS light and that glycine may facilitate the process of degradation. According to the literature, the most probable product of such degradation is *N*-furfuryl-5-sulfamoyl anthranilic acid (Figure 8b) [6,7].

Amiloride has a strong absorption at 360 nm showing its potential photoreactivity. When amiloride in water was irradiated at the level of 30 W/m^2 for 30 min, the degradation of *ca*. 50% was observed [23]. Photodegradation of amiloride was also observed in the pH range 4.5-11 [18]. In our experiment, photoreactivity of amiloride was observed in DSC scan as shifting the first endotherm from 130°C to 123°C, suggesting that amiloride components responsible for this process changed. As a result, we suppose that amiloride is photolabile in a solid state.

4 Conclusions

Three different methods, *i.e*. DSC, FT-IR, NIR as well as NIR with chemometric assessments were used to examine interactions of three diuretics, torasemide, furosemide and amiloride with eight excipients. It was found that these drugs showed interactions with citric acid, PVP, Mg stearate and glycine. Our results were mostly consistent, offering complementary information about the functional groups involved in the drug-excipient interactions. For these drugs, the hydrogen-bond type interactions with some of the excipients were postulated. In addition, other types of interactions were indicated, including accelerated degradation at high temperature and humidity. In addition, furosemide and amiloride, as individuals or in the mixture with glycine, showed the lack of stability under UV/VIS irradiation. Naturally, we realize that the mechanisms proposed for the observed interactions require further confirmation by additional methods, *e.g*. LC-MS. However, we can conclude that a selection of appropriate excipients for torasemide, furosemide and amiloride is a very important question for minimizing their degradation processes.

Acknowledgements: The paper was developed using the equipment purchased within the Project "The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic disease" within the Operational Program Development of Eastern Poland (2007-2013), priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

The Authors wish to thank Biofarm Sp. z o. o. (Poland) for kindly supplying torasemide pure substance.

Conflict of interest: Authors state no conflict of interest.

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