PRELIMINARY DETERMINATION OF HYDROLYTIC STABILITY OF A PYRROLE-BASED HYDRAZIDE AND ITS HYDRAZONE

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Abstract: A validated UV/VIS method for preliminary determination of the chemical stability and stability in close to physiological conditions of a model pyrrole hydrazide and its corresponding derivative, bearing susceptible to hydrolysis hydrazone group was developed. The evaluated substances were subjected to the influence of a variety of pH medias, representing the main physiological values of 37°C and corresponding pH values in the stomach (pH 2.0), blood (pH 7.4) and small intestine (pH 9.0). The chemical stability at strong alkali media of pH 13.0 was also evaluated. The hydrazide was found to be stable at all investigated conditions. The tested hydrazone was determined to be stable at pH of 7.4 and a temperature of 37°C and susceptible to hydrolysis at strong acidic (pH 2.0) and moderate alkali (pH 9.0) media at the same temperature. In addition, a decrease in the absorption at strong alkali media (pH 13.0) was observed, showing the compounds instability under these conditions.

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Keywords: pyrrole-hydrazide, hydrazone, stability, UV/VIS

Introduction

Pyrrole and its derivatives play a fundamental role in pharmaceutical and natural chemistry. Pyrrole is an aromatic five membered ring with the formula C4H5N, composed of four carbon atoms and one nitrogen atom. Pyrrole itself is not naturally occurring, but many of its derivatives are found in a variety of co-factors and natural products (Loudon, 2002; Jusélius, 2000; Cox, 2000).

In the recent years the synthesis, reactions and biological activities of pyrrole derivatives are of great interest of many researches (Jones, 1990). This is due to the fact that a lot of the marketed drugs possessing a pyrrole ring system have various biological properties such as antipsychotic, anti-tubercular, anti-inflammatory, antibacterial, antifungal, antiviral, anxiolytic, anticancer (leukemia, lymphomaandmyelofibrosisetc.), anti-hyperglycemic, antiprotozoal, antimalarial and many more (Bijev, 2010; Bhardwaj, 2015; Wilkerson, 1995; Lee, 2001; Vladimirova, 2016).

In addition, pyrrole and its derivatives are also found as components of many polymers, dyes and larger aromatic rings (Wurz, 2005; Piliego, 2010; Katritzky, 1989). Some of these compounds are also useful intermediates in the synthesis of biologically important naturally occurring alkaloids and synthetic heterocyclic derivatives (O'Hagan, 2000; Chinchilla, 2004).

The biological activity of the substances available as pharmaceuticals, agrochemicals and flavours or fragrances have to be efficiently delivered to their target site and released at a well-defined rate for optimal performance (Levrand, 2006).

As an important factor influencing this performance and the therapeutic activity of new compounds expressing various pharmacological effects is their stability at different physiological conditions, such as: body temperature of 37°C and physiological pH of 1.2 – 2.0 (in stomach), 7.4 (in blood plasma) and 8.5 – 9.0 (in small intestine) (Kong, 2008).

The strict correlation between the stability and the pharmacokinetic behavior in the body and to the conditions for the formulation, storage, occurrence of toxic effects associated with the degradation of products and so on is essential in the development of new biologically active substances.

In our previous determinations we have established that most pyrrole containing compounds derived by us are fairly stable in the neutral pH value found in the intestine but can be unstable at the lower pH value found in the stomach (Zajac, 2010). In this regard, early information for stability at different physiological conditions of the new compounds is of particular importance for subsequent processes of optimization and the selection of leading active structures. The obtained information may prevent unnecessary costs on developing products that subsequently prove to be unstable (Vladimirova, 2016).

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The aim of this study is to determine the chemical stability in strong alkali media of pH 13 and the stability under physiological conditions, like the temperature of 37°C and pH of 2.0 (stomach), 7.4 (blood plasma) and 9.0 (small intestine) (Georgieva, 2012). of new pyrrole-containing hydrazide (D_5) and its hydrazone (D_5a).

Selection of a model compound

Previously synthesized derivatives containing a pyrrole cycle, bearing a variety of functional groups as hydrazide, ethoxy-carbonyle, acetyl, carboxamide etc. have been evaluated for chemical stability and stability at close to physiological conditions. These experiments have demonstrated that the fully substituted pyrrole cycle, as well as the methyl groups connected with it are stable at moderate temperatures and in wide range of pH values (Kalia, 2008; Georgieva, 2012). Therefore, potential “vulnerable” groups susceptible to hydrolysis may be considered the ester, and to a lower extend the amide group and the formed after condensation hydrazone group (Jordanov, 1994; Kalia, 2008). Previous investigations with analogous compounds have already proven the ester group is considered above to be stable at the modeled physiological conditions (Georgieva, 2012). Therefore, for our investigations as groups standing out as substantial moieties likely to be susceptible to hydrolysis were considered the hydrazide and the corresponding hydrazone groups.

Thus, two model compounds bearing two hydrolytically susceptible groups (hydrazide and hydrazone) were selected: ethyl 5-(4-bromophenyl)-1-(1-hydrazinyl-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (D_5) and (E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(2-hydroxybenzylidene) hydrazinyl)-4-methyl-1 oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (D_5a).

A suitable method for preliminary evaluation of the possible degradation was selected. In this case this was UV/VIS spectrometry, since the model structures were in compliance with the ease of UV/VIS change measurements.

Methods

Synthesis of the model hydrazide

The investigated hydrazide was synthesized according to a general synthetic procedure, presented elsewhere (Tzankova, 2017).

Synthesis of the model hydrazone

For our investigations the model hydrazone was obtained through condensation of the relevant hydrazyde and salicylaldehyde according to a procedure described in (Georgieva, 2012). The synthesis was performed in an acetic acid medium by heating an equimolar ratio of the participating reagents. The target product was isolated in water, and further purified by recrystallization in ethanol.

Preparation of the buffers 2.0, 7.4, 9.0 and 13.0

The buffers were prepared according to a procedure enlisted in European Pharmacopoeia 7.0 (Eur. Ph. 7.0.).

Chemical stability evaluation

All compounds presented in this paper have been stored for 6 months at room temperature with access to air and light.

For the evaluation of the chemical stability an acetonitrile: buffer solution with pH 13.0 was prepared. The proper amounts of the model compounds were weighed and dissolved in the corresponding mixture of acetonitrile: buffer pH 13.0 (Solution 4) in a way that the concentration is in the range of 4.10^{-6} mol/l. The obtained solutions were kept at room temperature and stirred at 37ºC for a total time of 1440 min. Aliquot samples of 2 ml of the analyzed solutions were taken at definite time intervals (0, 30, 60, 90, 120, 180, 210, 240, 480 and 1440 min.) and the corresponding UV/VIS spectra were recorded. The absorbencies at 272 nm for D_5 and at 286 nm for D_5a were detected.

Hydrolytic stability evaluation

Due to the poor solubility of the evaluated compound D_5a in water, for the evaluation of the stability an acetonitrile: water solution was prepared at relevant ratio of acetonitrile: water = 1:50. For the purpose of this study, the necessary acetonitrile: buffer solutions were prepared at the same relevant ratio, in order to obtain the desired pH values, close to physiological. The proper amounts of the model compound were weighed and dissolved in the corresponding mixtures giving pH values of 2.0

(Solution 1), 7.4 (Solution 2) and 9.0 (Solution 3) in a way that the concentration is in the range of 4.10^{-6} mol/l. The obtained solutions were stirred at 37ºC for a total time of 1440 min. Aliquot samples of 2 ml of the analyzed solutions were taken at definite time intervals (0, 30, 60, 90, 120, 180, 210, 240, 480 and 1440 min.) and the corresponding UV/VIS spectra were recorded. The absorbencies at 272 nm for D_5 and at 286 nm for D_5a were detected.

Validation procedure

The developed UV/VIS method for stability evaluation was tested with respect to the validation parameters enlisted in the ICH Q2 (R1) guidelines (ICH), in means of precision, linearity, accuracy and selectivity.

Precision: Precision of the method was tested by analyzing six independent solutions from each of the tested product. The final results are reported as relative standard deviations (RSD %).

Linearity: Linearity was determined within the range of 25-200 μg/mL for both the hydrazide D_5 and its hydrazone D_5a. Calibration curves were created using 8 points covering 8 different concentrations of the tested compounds over the evaluated concentration range. A linear regression was used to process the calibration data.

Accuracy: The solutions for measurement were prepared using a placebo and stock solution of the tested structures. Each solution was measured on the UV/VIS spectrometer three times. Accuracy is reported as a parameter recovery with relative standard deviations.

Selectivity: The selectivity was determined by comparing the UV/VIS spectra for the solutions of the tested **D_5** and **D_5a** with the solutions of the used solvents alone and in mixture.

Results and discussion

Validation of the developed UV/VIS analytical procedure

The method was validated according to ICH Q2 (R1) guidelines (ICH). The method's precision, linearity, accuracy and selectivity were evaluated during the validation. The obtained parameters for the measured absorptions at 286 nm are presented on **Table 1**.

Table 1: Validation parameters for compounds D_5 and D_5a.

	D_5	**D_5a**	**Criterion**
Repeatability (% RSD)*	0.35	0.25	$X < 1\%$
Precision (% RSD)#	2.0	2.5	$X < 5\%$
Linearity (correlation coefficient)§	0.996	0.997	R > 0.990
Accuracy (%)#	100.5	99.95	$X = 100 \pm 5\%$
Selectivity	No interference	No interference	No interference

*Six measurements.
Two samples, three measurements of each solution.
§ At 25, 50, 75, 100, 150, 175 and 200 μg/mL concentration level.
% RSD: Relative standard deviation in %

Source: Author

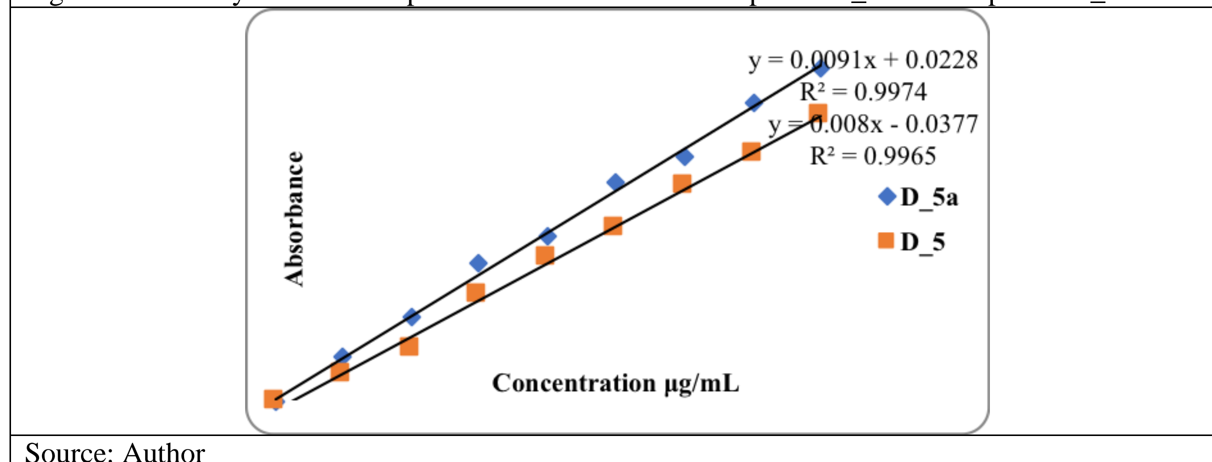
Precision*:* The calculated RSD values for D_5 and D_5a for the assessment of the precision are 2-2.5 %, confirming that the method is precise.

Linearity*:* The correlation coefficients of linearity are 0.996 for D_5 and 0.997 for D_5a. The values indicate good correlation between the measured absorbencies at 272 nm and 286 nm for D_5 and D_5a, respectively in the range of concentrations studied. A representative graph of the linearity is presented on Figure 1.

Accuracy*:* The method was found to be accurate with recoveries of 99.95%–100.5%

Selectivity*:* No interferences of the present solvents were observed which indicates that the method is selective and could be used for further analysis.

Figure 1: Linearity of the developed UV/VIS method for compound D_5 and compound D_5a

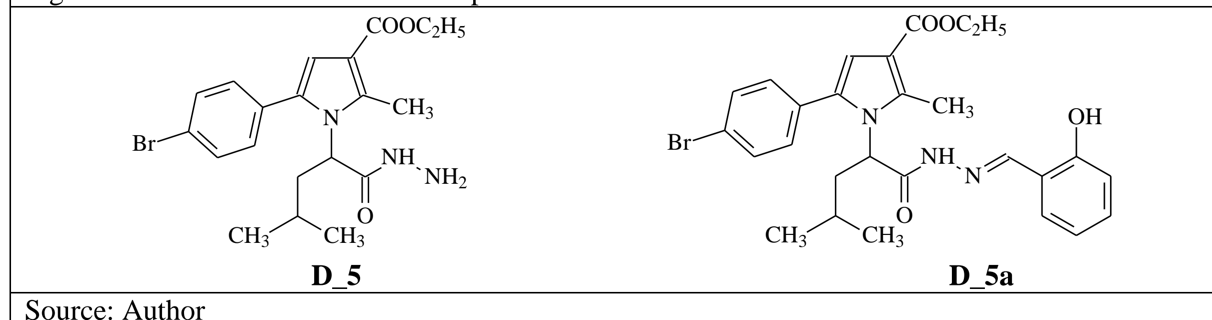


Source: Author

Stability investigations

In an attempt to determine the chemical stability and the stability at close to physiological conditions the synthesized derivatives D_5 and D_5a (Figure 2) were followed for hydrolytic decomposition under physiological temperature of 37 °C and at a wide range of pH varying from 2.0, 7.4 and 9.0, namely the physiological pH in the stomach, blood plasma and intestines, respectively up to pH 13.0 for chemical stability. We considered as the most probable change in compound D_5a to be cleavage of the characteristic hydrazone group -CH=N-. For compound D_5 hydrolysis in the $-CONHNH_2$ group or the ester group at 3rd position in the pyrrole ring was expected.

Figure 2: Structures of the model compounds



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Chemical stability

Chemical stability is defined as the possibility of a substance to sustain in response to a change or decomposition as a result of any internal reaction, action of air, humidity, heat, light, pressure, etc. All evaluated compounds have been stored for 6 months at room temperature with access to air and light. No change in the physical and chemical properties of the investigated compounds was observed under these conditions. Thus, the tested compounds may be considered as chemically stable, when stored.

In addition, the chemical stability was investigated in a strong alkali media of pH 13.0 and at two temperatures: room temperature and 37°C.

Physiological stability

An important factor influencing the performance of the molecules in the organism is their hydrolytic stability at physiological conditions, such as: body temperature of 37 °C and physiological pH of 2.0 (in stomach), 7.4 (in blood plasma) and 9.0 (in intestine) (Georgieva, 2012).

For both types of stability evaluation, four solutions were prepared according to the above mentioned recipe. The obtained values for the corresponding absorbances at 272 nm for compound D_5 and at 286 nm for compound D_5a for the physiological conditions were recorded and are presented in Table 2, Table 3 and Table 4 respectively.

Some previous investigations with analogous hydrazides have shown that the followed $CONHNH_2$ group and the ester group at the 3rd position in the pyrrole ring are stable in the above mentioned

physiological conditions (Vladimirova, 2009, Georgieva, 2012). This was confirmed by the data obtained in our investigation.

Table 2: Measured absorbances for Solution 1 at the cited time intervals

Aliquot №	Time interval (min)	**D_5** Absorbance at 272 nm	**D_5a** Absorbance at 286 nm
1	0	0.11443	0.33128
2	30	0.106088	0.29166
3	60	0.10175	0.25713
4	90	0.10176	0.23923
5	120	0.10362	0.22583
6	180	0.10672	0.21248
7	210	0.10982	0.21693
8	240	0.11522	0.20796
9	480	0.10726	0.20570
10	1440	0.12575	0.17795

Source: Author

Table 3: Measured absorbances for Solution 2 at the cited time intervals

Aliquot №	Time interval (min)	**D_5** Absorbance at 272 nm	**D_5a** Absorbance at 286 nm
1	0	0.14081	0.50609
2	30	0.13896	0.50669
3	60	0.14532	0.50715
4	90	0.18207	0.49971
5	120	0.15393	0.51459
6	180	0.15186	0.51498
7	210	0.17661	0.50738
8	240	0.17113	0.49896
9	480	0.14941	0.50963
10	1440	0.20096	0.50186

Source: Author

Table 4: Measured absorbances for Solution 3 at the cited time intervals

Aliquot №	Time interval (min)	**D_5** Absorbance at 272 nm	**D_5a** Absorbance at 286 nm
1	0	0.14758	0.307981
2	30	0.13533	0.29271
3	60	0.13687	0.27589
4	90	0.13467	0.27254
5	120	0.13249	0.25426
6	180	0.13736	0.23357
7	210	0.14342	0.23667
8	240	0.14207	0.22194
9	480	0.13419	0.21922
10	1440	0.13295	0.18620

Source: Author

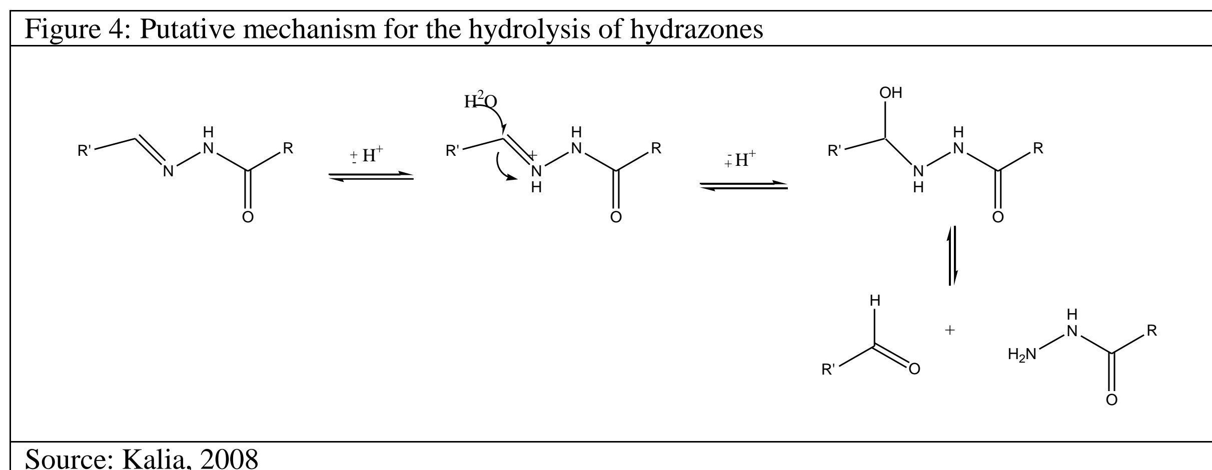
In the structure of the evaluated model of hydrazone D_5a, two hydrolytically susceptible groups are available: the ester group at the 3rd position in the parental hydrazide, and the newly formed hydrazone group. From the above obtained results for the initial hydrazide D_5 was determined that an object of hydrolysis in D_5a would be the targeted hydrazone group.

There are a number of sources confirming our suggestion that the hydrazone hydrolysis is connected mainly with cleavage of the C=N double bond from the hydrazone group and release of the parental

hydrazide and corresponding carbonyl compound (aldehyde or ketone) (Kalia, 2008; Kovaříková, 2006; Love, 1963).

The mechanism that may be considered as most probable may be the $C^1=N^1$-NH-CO-R hydrolysis that entails the protonation of N^1 (Scheme 2). The resultant protonated species would be highly susceptible to hydrolysis because of the enhanced electrophilicity of C^1 (Kalia, 2008).

Figure 4: Putative mechanism for the hydrolysis of hydrazones



Source: Kalia, 2008

In the performed investigations a small decrease in the absorptions for compound D_5a in acidic (pH 2.0) and alkali (pH 9.0) media is observed. This led us to conclude that this substance is susceptible to hydrolysis at these conditions.

The data indicates that a hydrolytical cleavage of the hydrazone C=N double bond is expected, with the formation of the parental hydrazide and the corresponding benzaldehyde. The identification of the possible degradation products is a subject of further analysis, which will be published additionally.

Conclusion

A validated UV/VIS spectrometrical method was applied for the preliminary evaluation of the stability of a model pyrrole containing hydrazide and its hydrazone. The analysis was followed at conditions close to physiological with pH values of 2.0 (in stomach), 7.4 (in blood plasma) and 9.0 (in small intestine) and temperature of 37 C. The susceptibility of the model compounds to hydrolysis in strong alkali media was also studied. Compound D_5 was found to be stable at all conditions. The tested model hydrazone D_5a was determined to be sensitive to hydrolytic decomposition in aqueous media and pH values of 2.0 and 9.0 and temperature of 37°C, resulting probably in the splitting of the hydrazone bond. In addition, a decrease in the absorption in the strong alkali media (pH 13.0) was also observed, which showed that the investigated compounds are sensible under these conditions.

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