PHARMACIA

Pharmacia 66(3): 127–134
DOI 10.3897/pharmacia.66.e47035

Research Article

Development and validation of RP-HPLC method for stability evaluation of model hydrazone, containing a pyrrole ring

Diana Tzankova[1], Lily Peikova[1], Stanislava Vladimirova[2], Maya Georgieva[1]

1 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*
2 *Department of Organic Chemistry and Fuels, University of Chemical Technology and Metallurgy, Sofia, Bulgaria*

Corresponding author: *Lily Peikova* (lily_peikova@yahoo.com)

Received 11 October 2019 ♦ **Accepted** 8 November 2019 ♦ **Published** 12 November 2019

Citation: Tzankova D, Peikova L, Vladimirova S, Georgieva M (2019) Development and validation of RP-HPLC method for stability evaluation of model hydrazone, containing a pyrrole ring. Pharmacia 66(3): 127–134. https://doi.org/10.3897/pharmacia.66.e47035

Abstract

RP-HPLC method with UV detection was developed and validated for determination of the chemical stability and stability in close to physiological conditions of a model pyrrole hydrazone ethyl 5-(4-bromophenyl)-1-(1-(2-(4-hydroxy-3-methoxybenzylidene) hydrazineyl)-4-methyl-1-oxopentan-2-yl)-2-methyl-1*H*-pyrrole-3-carboxylate (**D_5d**), containing susceptible to hydrolysis hydrazone group. The evaluated substance was subjected to the influence of a variety of pH , 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). Chemical stability in a highly alkaline medium with a pH of 13.0 was also evaluated. The hydrazone I tested was found to be stable at pH 7.4 and pH 9.0 and 37 ° C and hydrolyzed under strong acidic (pH 2.0) and highly alkaline media (pH 13.0) and at the same temperature.The products of hydrolysis were identified to be the initial hydrazide and aldehyde, pointing the hydrazone group as most liable.

Keywords

pyrrole-hydrazide; pyrrole-hydrazone; stability; RP-HPLC, validation

Introduction

Novel pyrrole containing hydrazones were synthesized and extensively investigated from the view point of novel drug development. Many previous studies (*in vitro* and *in vivo*) have indicated a wide variety of their remarkable biological properties such as anticancer (Alsante et al. 2001, Kovarıkova et al. 2008) antioxidant, anti-tuberculosis, (Richardson et al. 1989, Kovarıkova, et al. 2006, Vladimirova et al. 2016) anti-inflammatory (Svirskis et al. 2011) antidepressant (Tzankova et al. 2019a), antihypertensive, (Shaalan and Belal 2010, Grosjean et al. 1999) antimicrobial (Georgieva et al. 2012, Tzankova et al. 2018, 2019b, Kalia and Ronald 2008, Kovaříková et al. 2006) and anticonvulsants (Love 1963, Xia et al. 2008, Alqasoumi et al. 2009, Mohareb et al. 2010, Georgieva et al. 2012, Lessigiarska et al. 2012, El-Tombary and El-Hawash 2014, Kareem et al. 2015, Puskullu et al. 2016, Tzankova et al. 2018) activity. These hydrazones seem to be promising drug candidates with potential to be used in the treatment of several human pathologies (Bijev and Georgieva 2010, Navakoski et al. 2011, Kajal et al. 2014, Georgieva et al. 2017).

The process of novel drug development includes stability assessment of any promising drug candidate. Environmental factors, such as heat, light, moisture as well as the inherent chemical susceptibility of compounds to hydroly-

sis or oxidation play an essential role in drugs stability. Stability tests are used to define storage and handling conditions. Using of extreme external conditions in the stability studies (stress tests) helps revealing and identifying the likely degradation products (Ajani et al. 2010, de Oliveira et al. 2011, Leal et al. 2012, Yurttaş et al. 2013, Vaigunda et al. 2017) Several analytical methods like UV-VIS and HPLC (Dimmock et al. 2000) are reported in the literature for the identification and quantification of degradation of different pharmaceutical substances in various media.

The HPLC method with diode array detection (DAD) is widely used for determination of stability of pharmaceutical compounds The (2,4-dinitrophenyl) hydrazones of carbonyls are separated by liquid chromatography and detected by ultraviolet spectroscopy (diode array detector).

Selection of a model compound

From all previously synthesized compounds for purpose of the current study were used the initial hydrazide are ethyl 5-(4-bromophenyl)-1-(1-hydrazinyl-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (**D_5**) bearing the hydrolytically susceptible hydrazide group the corresponding aldehyde 4-hydroxy-3-methoxybenzaldehyde (**d**) and the product of their interaction – the hydrazone (*E*)-ethyl 5-(4-bromophenyl)-1-(1-(2-(4-hydroxy-3-methoxybenzylidene)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-2-methyl-1*H*-pyrrole-3-carboxylate (**D_5d**), bearing the hydrolytically susceptible hydrazone group. Some previous investigations with analogous hydrazides have shown that the followed $CONHNH_2$ group and the ester group at 3rd position in the pyrrole ring are stable at the evaluated physiological condition. Thus of an interest to our investigation to hydrolysis was subjected only compound **D_5d**.

Experimental part

Chemicals and reagents

The model compounds were synthesized by Paal-Knorr condensation and characterized as described previously (Tzankova et. al. 2019a; Tzankova et. al. 2019b) All the chemicals and reagents used as starting materials were purchased from Merck (Darmstadt, Germany).

The necessary products for preparation of the mobile phase and buffers were obtained from Sigma-Aldrich (Steinheim, Germany).

Chromatographic system and conditions

The chromatographic system UltiMateDionex 3000 SD, Chromeleon 7.2 SR3 Systems, Thermo Fisher Scientific Inc was applied. The analysis was achieved on an analytical column 250 mm x 4.6 mm (Purospher® STAR, RP-18, 5 μm) protected with a guard column (Hibar®, RT 125-4). The column oven was conditioned at 25°C. The mixture of an acetonitrile, phosphate buffer pH 4.0 and methanol in a ratio 60:30:10 (v/v/v) was used as a mobile phase. The flow rate was 1.0 ml/min and the detector was set up to 272 nm. The injection volume was 20 μl with analysis time of 15 minutes.

Preparation of buffer component of mobile phase

Measured amount of 5.04 g of sodium hydrogen phosphate dihydrate and 3.01 g of potassium dihydrogen phosphate dihydrate were dissolved in 1000 ml of ultrapure water. Glacial acetic acid was used to adjust the pH to 4.0. The mobile phase buffer was filtered through a membrane filter (0.20 μm) using a Millipore glassfilter holder. The mobile phase buffer was used immediately after preparation or stored in the refrigerator in closed borosilicate glassbottles for a maximum of 24 hours.

Preparation of buffers included in the stability evaluation

Preparation of buffer pH 2.0

Measured amount of 6.57 g of KCl (dissolved in water free from CO_2) and 119.0 ml of hydrochloric acid (0.1 mol/l) were dissolved in 1000 ml distilled water.

Preparation of buffer pH 7.4

Measured amount of 2.38 g of sodium hydrogen phosphate dihydrate, 0.19 g of potassium dihydrogen phosphate dihydrate and 8.0 g NaCl were dissolved in 1000 ml distilled water.

Preparation of buffer pH 9.0

Solution A – Measured amount of 6.18 g boric acid was dissolvedin 0.1 mol/l KCl. The obtained mixture was diluted up to 1000 ml with the same solvent.

Solution B – 0.1 mol/l NaOH.

Buffer pH 9.0 was prepared, where 1000 ml ofsolution. A are mixed with 420.0 ml of solution B.

Preparation of buffer pH 13.0

A standard buffer with pH 13.0 (KCl/ NaOH) was used.

Preparation of samples for analysis for determination of stability at pH = 2.0, 7.4, 9.0 and 13.0

Measured amount of 0.0010 g sample of the analyzed compound was weighed and dissolved in 5 ml acetonitrile. The obtained mixture was diluted up to 50 ml with the corresponding buffers of pH 2.0, pH 7.4, pH 9.0 and pH 13.0. The obtained solutions were stirred at 37°C for a total time of 240 min. An aliquot 20 μl samples were drawn and injected into the HPLC system at the defined time intervals.

Mobile phase composition

Mobile phase, consisting of CH_3CN/phosphate buffer pH 4.0/ CH_3OH = 60/30/10 (v/v/v) was prepared and applied.

Method development

In order to achieve good separation and proper chromatographic conditions for stability assessment, we have endeavored to obtain chromatographic conditions which would allow the determination of as many peaks as possible from the set of test samples. The most common separation conditions include solvent type, mobile phase composition and pH of the medium, column type and column temperature.The experimental conditions for stability analysis were achieved by planned / systematic study of parameters including the pH of the medium, components included in the mobile phase and ratios, gradient, flow rate, temperature, sample volume, injection volume and solvent type.

Method validation

The developed RP-HPLC method was tested with respect to following validation parameters: specificity, precision, linearity, accuracy and selectivity.

Specificity and selectivity

Specificity of the chromatographic analytical procedure is the ability to measure the analyte response in the presence of all potential sample components such as the starting materials, intermediates in the synthesis, and inactive ingredients in the formulated products, and the degradation products. The selectivity of the method was evaluated by comparing the solution of the model compound (**D_5d**) with the solution of the corresponding hydrazide, aldehyde and used mobile phase alone and in mixture.

Precision

Precision is expressed as the standard deviation of the analytical results when the analysis is carried out in a laboratory by an operator using equipment over a relatively short time span. For precision of the method **six** independent sample solutions from evaluated hydrazone were used. Each sample was tested in triplicate. The final results are reported as relative standard deviations (RSD %).

Linearity

The linearity of an analytical method can be defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a given range. The linearity of the method was determined in a concentration range of 5 - 40 μg/mL for hydrazone **D_5d**. An eight points covering the eight different concentrations of the analyzed compound over the evaluated concentration range were used to create the calibration curve. For processing the calibration data was used linear regression.

Accuracy

Accuracy is the closeness of the analytical results obtained by the analyses to the true values, and usually presented as a percent of nominal. The analyzed solutions were prepared using a placebo and stock solution of the tested structure. Accuracy is reported as a parameter recovery with relative standard deviations calculated from the analysis of six solutions prepared from the compound. Each solution was tested in triplicate.

The aim of this study is to develop, validate and apply a simple RP-HPLC method for stability determination of pyrrole-containing derivatives.

Results and discussion

Specificity and stability indicating study

Specificity is the ability of the analytical method to measure the active ingredient response in the presence of other excipients and its potential degradants. Forced degradation was carried out to evaluate the specificity and stability-indicating properties of the method, by exposing samples of the substance to stress conditions of hydrolysis, oxidation, photodegradation, and thermal degradation.

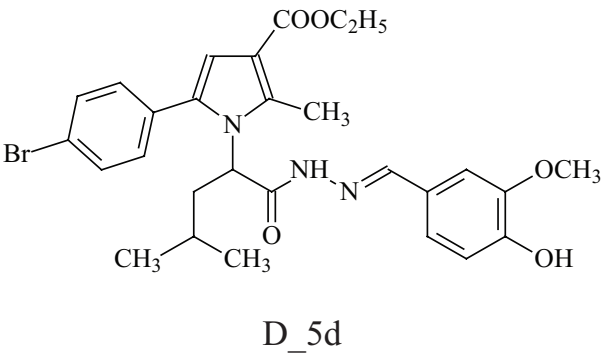
Stress testing of the substance was performed to induce force degradation and determine degradation pathways and help evaluate the stability of the compound and also validate specificity of the analytical procedures.

Selection of a model compound.

There are a number of data 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)

A previously developed and applied by us UV/VIS method for preliminary evaluation of the stability of this group of compounds determined that liable to hydrolysis is expected to be the corresponding hydrazone group in the tested model compounds

Thus in order to evaluate and identify the products of hydrolysis from previously synthesized by us pyrrole containing hydrazones, as model compound was selected compound **D_5d**. The structure of the model hydrazone is presented on Figure 1.



D_5d

Figure 1. Structure of the selected model compound.

Its stability was determined at different pH (2.0, 7.4, 9.0 and 13.0), close to physiological and stressful conditions of pH 13.0 and at two temperatures – (25 °C and elevated (37 °C).

Method development and optimization

With regard to the physical and chemical properties of the analytes and the information obtained from the literature, analytical method was developed to select a preliminary reversed phase RP-HPLC chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For that, series of trials were performed, such as different compositions of mobile phase and different types of stationary phase and column lengths, with different pH values and buffering agents.

The performed experiments demonstrated that including acetonitrile as component of the mobile leads to improvement in the separation and formation of well developed Gaussian type peaks. Different compositions of solvents were analyzed, containing methanol, acetonitrile, buffer with pH of 2.0, 7.4 and 4.0 in various relations. As most suitable for achieving good separation and suitable peak forms was determined to be a mobile phase, consisting of CH_3CN/phosphate buffer pH 4.0/ CH_3OH in 60/30/10 (v/v/v). In addition the flow rate, the temperature and the wavelength were also varied as follows: the flow rate was evaluated in the range of 0.9 to 1.2 ml/min. The flow rate of 1.0 ml/min was found to be most appropriate. A temperature of 25°C was found to be suitable for the good separation. The preliminary UV/VIS method developed by us identified 272 nm as adequate wavelength.

Thus the performed analysis determined the following chromatographic conditions, which were further applied for identification and determination of stability of the tested hydrazone **D_5d**:

- Mobile phase: CH_3CN/phosphate buffer pH 4.0/ CH_3OH in 60/30/10 (v/v/v)
- Isocratic flow with flow rate of 1.0 ml/min
- Column temperature of 25°C
- Wavelength of 272 nm

Standard solutions of the analyzed hydrazone (**D_5d**) and the expected initial hydrazide (**D_5**) and aldehyde (**d**) were analyzed by the developed method for identification of the corresponding retention times. Under the discussed chromatographic conditions was found thet the retention times for the tested hydrazone (**D_5d**), the initial hydrazide (**D_5**) and aldehyde (**d**) are as follows: t_R = 6.800 (**Figure 2**), t_R = 4.387 (**Figure 3**) and t_R = 1.387 (**Figure 4**), respectively.

The obtained under these conditions separation is presented on **Figure 5**.

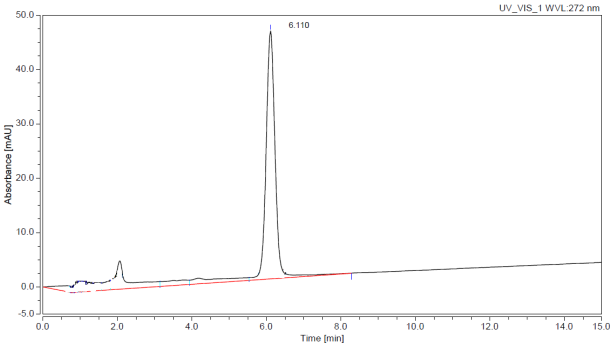


Figure 2. Chromatogram of standard solution of the analyzed hydrazone **D-5d** (t_R = 6.110).

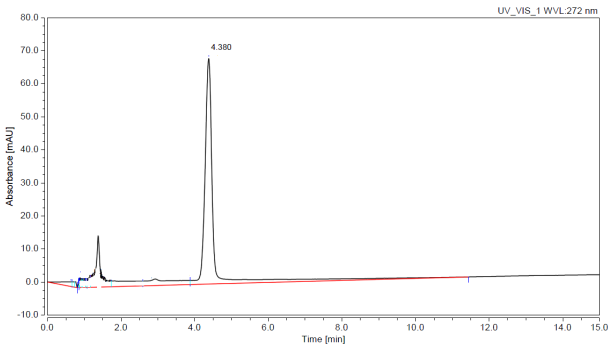


Figure 3. Chromatogram of standard solution of the hydrazide **D-5** (t_R = 4.380) as possible degradation product.

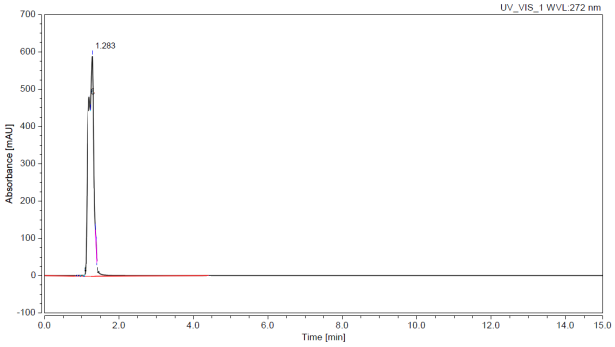


Figure 4. Chromatogram of standard solution of the aldehyde **d** (t_R = 1.283) as possible degradation product.

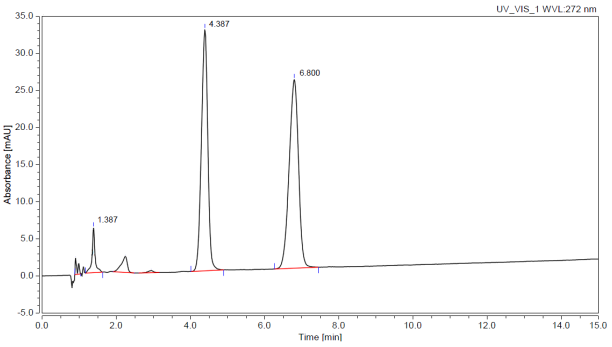


Figure 5. Chromatogram of the separated mixture of the analyzed hydrazone **D-5d** (t_R = 6.800) and its possible degradation products – the hydrazide **D-5** (t_R = 4.387) and the corresponding aldehyde **d** (t_R = 1.387).

Method validation

Specificity

No significant interfering peaks (peak area >0.1%) was observed at the retention time of the analyzed hydrazone and mixtures in blank solution. In addition, no evidence of co-elution was noted using peak purity analysis for the tested compounds and mixtures.

Linearity

The linearity of the method was observed in the concentration range of 5 µg/mL to 40 µg/mL for **D_5d** demonstrating its suitability for analysis. The goodness of fit evaluated through the correlation coefficient r^2 was found to be 0.9980, indicating a linear relationship between the concentration of analyte and area under the peak, as shown on **Figure 6**.

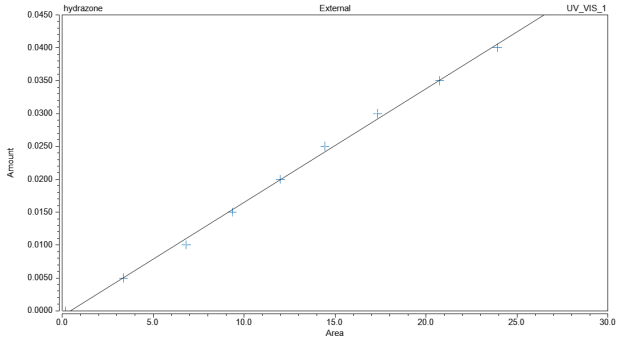


Figure 6. Linearity of the developed RP-HPLC **D_5d** stability indicating method.

Accuracy

The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy testing showed that the method is accurate within the acceptable limits. The percentage recovery and RSD were calculated for all active ingredients; all the results are within limits. Acceptable accuracy was within the range of 99.98% to 100.4% recovery and not more than 2.0% RSD, as demonstrated in **Table 1**.

Table 1. Evaluation of the accuracy of the method developed in this study.

Compound	Spiked level (µg/mL)	Replicate number	Recovery (µg/mL)	% mean recovery	% RSD
D_5d	5	1	5.05	100.4	0.72
		2	4.98		
		3	5.03		
	20	1	19.99	100.2	0.16
		2	20.04		
		3	19.98		
	40	1	40.07	99.98	0.23
		2	39.89		
		3	40.02		

Precision

Precision of an analytical method is defined as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation. The results of repeatability and intermediate-precision testing showed that the method is precise within the acceptable limits.The RSD was calculated and was found that the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in **Table 2**.

Table 2. Evaluation of precision of the method developed in this study.

Intermediate precision (ruggedness)	
Replicate number	**Recovery (µg/mL)**
1	19.99
2	20.01
3	20.03
4	19.98
5	20.04
6	19.87
Mean recovery	**19.99**
% RSD	**0.31**

Limit of Detection and Limit of Quantification (LOD and LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision.The method showed a LOD of 0.20 µg/mL and a LOQ of 0.40 µg/mL.

Chemical stability

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

In addition the chemical stability was investigated at strong alkali media of pH 13.0 and at two temperatures: room temperature and temperature of 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 et al. 2012)

The selected model compound was subjected to analysis following the requirements for physiological stability

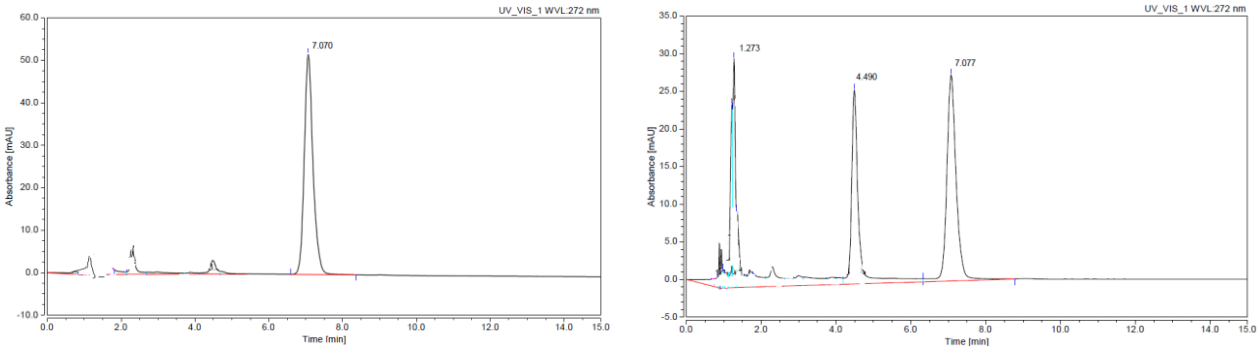
evaluation. The tested **D_5d** was dissolved in the corresponding buffers, mimicking physiological conditions and stirred at 37°C for 240 min.

For both types of stability evaluation four solutions were prepared according to the above mentioned recipe. The obtained chromatograms for the tested **D_5d** for the physiological conditions were recorded and are presented on Figure 7 and Figure 8 respectively.

In the performed investigations an appearance of new peaks, corresponding to the retention times of the initial hydrazide and the corresponding aldehyde are observed in the chromatogram of **D_5d** in acidic (pH 2.0) (**Figure 7**) media. This led us to conclude that this substance is susceptible to hydrolysis at these conditions and as a result are formed the initial reagents.

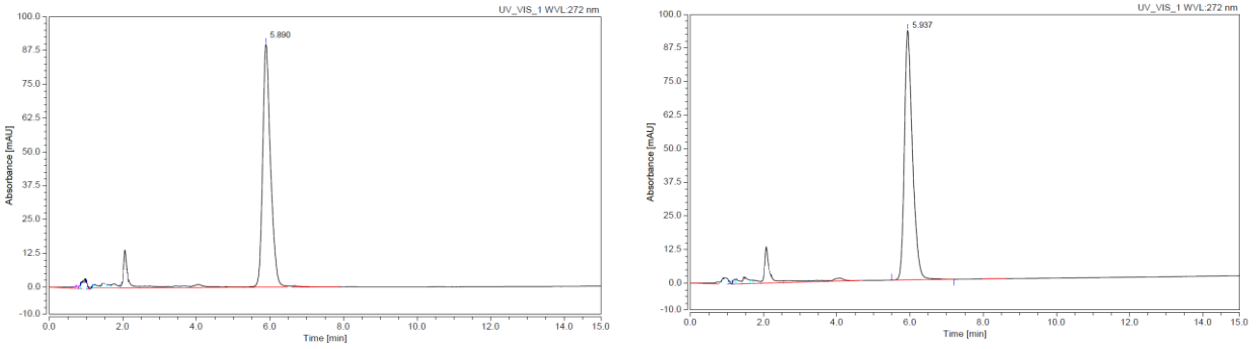
No additional peaks appeared in the chromatograms of the analysis performed in the neutral buffer of pH = 7.4 and alkali buffer of pH 9.0 (**Figure 8**) and temperature of 37°C for the evaluated period of time, which led us to conclude that the structure is stable at these conditions.(33–35).

In addition the chemical stability was also evaluated in the same manner by following the same procedure, using buffer of pH = 13.0. The corresponding chromatogram is presented on Figure 9.



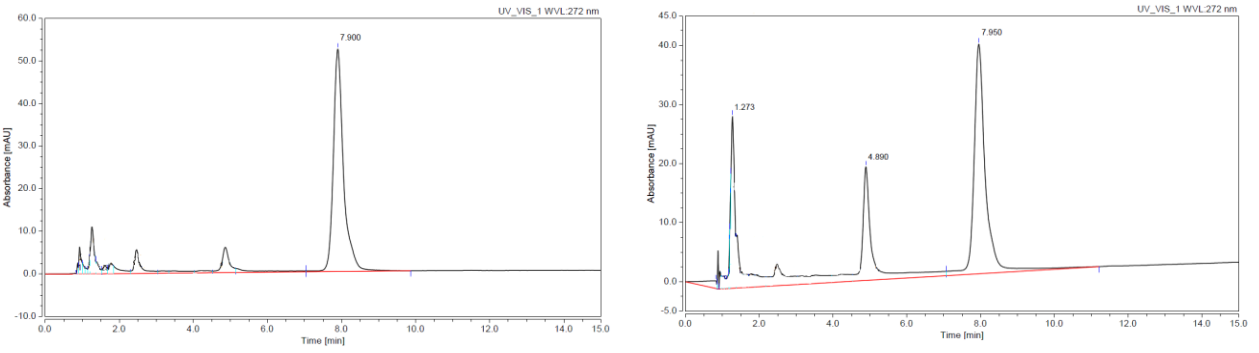
A B

Figure 7. Chromatograms indicating the behavior of **D_5d** in the presence of buffer with pH 2.0 and at 37°C at 0th min (**A**) and at 30th min (**B**).



A B

Figure 8. Chromatograms indicating the behavior of **D_5d** in the presence of buffer with pH 9.0 and at 37°C at 0th min (**A**) and at 210th min (**B**).



A B

Figure 9. Chromatograms indicating the behavior of **D_5d** in the presence of buffer with pH 13.0 and at 37°C at 0th min (**A**) and at 30th min (**B**).

The chromatogram shows appearance of new peaks at the retention times of the initial hydrazide and the corresponding aldehyde. This shows that the structure is liable to hydrolysis at strong alkali media. The result is cleavage of the hydrolytically liable hydrazone group, as suggested previously.

Conclusion

A fast, simple, accurate, precise, and linear stability indicating HPLC method has been developed and validated for the simultaneous analysis of pyrrole hydrazones and its degradation products. The method is stability indicating and reliable to detect and quantify any potential degradation in the tested compounds during stability studies and can be used for routine quality control analysis. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions. The method indicates that the tested model hydrazone **D_5d** is sensitive to hydrolytic decomposition in aqueous media and pH values of 2.0 and 9.0 and temperature of 37°C, resulting in the splitting of the hydrazone bond and formation of the initial hydrazide and corresponding aldehyde. In addition a liability at strong alkali media (pH 13.0) is also observed, witnessing for sensibility under these conditions.

References

Alsante KM, Friedmann RC, Hatajik TD, Lohr LL, Sharp TR, Snyder KD, Szczesny EJ (2001) Degradation and impurity analysis for pharmaceutical drug candidates. In: Ahuja S, Scypinsk S (Eds) Handbook of Modern Pharmaceutical Analysis. Separation Science and Technology 3: 85–172. https://doi.org/10.1016/S0149-6395(01)80006-4

Alqasoumi SI, Ghorab MM, Ismail ZH, Abdel-Gawad SM, El-Gaby MS, Aly HM (2009) Novel antitumor acetamide, pyrrole, pyrrolopyrimidine, thiocyanate, hydrazone, pyrazole, isothiocyanate and thiophene derivatives containing a biologically active pyrazole moiety. Arzneimittelforschung 59(12): 666–671. https://doi.org/10.1055/s-0031-1296457

Ajani OO, Obafemi CA, Nwinyi OC, Akinpelu DA (2010) Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives. Bioorganic & Medicinal Chemistry 18(1): 214–221. https://doi.org/10.1016/j.bmc.2009.10.064

Bijev A, Georgieva M (2010) Pyrrole-based hydrazones synthesized and evaluated in vitro as potential tuberculostatics. Letters in Drug Design & Discovery 7(6): 430–437. https://doi.org/10.2174/157018010791306588

Dimmock JR, Vashishtha SC, Stables JP (2000) Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds. European Journal of Medicinal Chemistry 35(2): 241–248. https://doi.org/10.1016/S0223-5234(00)00123-9

El-Tombary AA, El-Hawash SA (2014) Synthesis, antioxidant, anticancer and antiviral activities of novel quinoxaline hydrazone derivatives and their acyclic C-nucleosides. Medicinal Chemistry 10(5): 521–32. https://doi.org/10.2174/1573406411309666006 9

Georgieva M, Bijev A, Nikolova I, Danchev N (2012) Determination of Hydrolytic stability and acute toxicity of previously synthesized pyrrole-based hydrazones. Pharmacia 59(1): 10–16. http://bsphs.org/wp-content/uploads/2012/12/Georgieva.pdf

Georgieva M, Tzankova D, Vladimirova S, Bijev A (2017) CBU International Conference on Innovations in Science and Education 5: 1083–1091. https://doi.org/10.12955/cbup.v5.1075

Grosjean E, Green PG, Grosjean, D (1999) Liquid chromatography analysis of carbonyl (2, 4-dinitrophenyl) hydrazones with detection by diode array ultraviolet spectroscopy and by atmospheric pressure negative chemical ionization mass spectrometry. Analytical Chemistry 71(9): 1851–1861. https://doi.org/10.1021/ac981022v

Kajal A, Bala S, Sharma N, Kamboj S, Saini V (2014) Therapeutic potential of hydrazones as anti-inflammatory agents. International journal of medicinal chemistry 2014: e 761030. https://doi.org/10.1155/2014/761030

Kalia J, Ronald TR (2008) Hydrolytic stability of hydrazones and oximes. Angewandte Chemie International Edition 47(39): 7523–7526. https://doi.org/10.1002/anie.200802651

Kareem HS, Ariffin A, Nordin N, Heidelberg T, Abdul-Aziz A, Kong K W, Yehye WA (2015) Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3, 4, 5-trimethoxy benzyl moiety. European Journal of Medicinal Chemistry 103: 497–505. https://doi.org/10.1016/j.ejmech.2015.09.016

Kovaříková P, Mokrý M, Klimeš J, Vávrová K (2006) HPLC study on stability of pyridoxal isonicotinoyl hydrazone. Journal of Pharmaceutical and Biomedical Analysis 40(1): 105–112. https://doi.org/10.1016/j.jpba.2005.06.021

Kovaříková P, Mrkvičková Z, Klimeš J (2008) Investigation of the stability of aromatic hydrazones in plasma and related biological material. Journal of Pharmaceutical and Biomedical Analysis 47(2): 360–370. https://doi.org/10.1016/j.jpba.2008.01.011

Leal CM, Pereira SL, Kümmerle AE, Leal DM, Tesch R, de Sant'Anna CM, Fraga CAM, Barreiro EJ, Sudo RT, Zapata-Sudo G (2012) Antihypertensive profile of 2-thienyl-3, 4-methylenedioxybenzoylhydrazone is mediated by activation of the A2A adenosine receptor. European Journal of Medicinal Chemistry 55: 49–57. https://doi.org/10.1016/j.ejmech.2012.06.056

Lessigiarska I, Pajeva I, Prodanova P, Georgieva M, Bijev A (2012) Structure-Activity Relationships of Pyrrole Hydrazones as New Anti-Tuberculosis Agents. Medicinal Chemistry 8: 462–473. https://doi.org/10.2174/1573406411208030462

Mohareb RM, Fleita HD, Sakka OK (2010) Novel synthesis of hydrazide-hydrazone derivatives and their utilization in the synthesis of coumarin, pyridine, thiazole and thiophene derivatives with antitumor activity. Molecules 16(1): 16–27. https://doi.org/10.3390/molecules16010016

de Oliveira KN, Costa P, Santin JR, Mazzambani L, Bürger C, Mora C, Nunes RJ, de Souza MM (2011) Synthesis and antidepressant-like activity evaluation of sulphonamides and sulphonyl-hydrazones. Bioorganic & Medicinal Chemistry 19(14): 4295–4306. https://doi.org/10.1016/j.bmc.2011.05.056

Puskullu MO, Shirinzadeh H, Nenni M, Gurer-Orhan H, Suzen S (2016) Synthesis and evaluation of antioxidant activity of new quinoline-2-carbaldehyde hydrazone derivatives: bioisosteric melatonin analogues. Journal of Enzyme Inhibition and Medicinal Chemistry 31(1): 121–125. https://doi.org/10.3109/14756366.2015.1005012

Richardson D, Vitolo LW, Bakers E, Webb J (1989) Pyridoxal isonicotinoyl hydrazone and analogues. Biology of Metals 2(2): 69–76. https://doi.org/10.1007/BF01129203

Svirskis D, Travas-Sejdic J, Garg S (2011) A stability indicating HPLC method for the determination of electrochemically controlled release of risperidone. Journal of Chromatographic Science 49(10): 780–785. https://doi.org/10.1093/chrsci/49.10.780

Shaalan RA, Belal TS (2010) HPLC-DAD stability indicating determination of nitrofurazone and lidocaine hydrochloride in their combined topical dosage form. Journal of chromatographic science 48(8): 647–653. https://doi.org/10.12955/cbup.v6.1314

Tzankova D, Peikova L, Georgieva M (2018) Preliminary determination of hydrolytic stability of a pyrrole-based hydrazide and its hydrazone. CBU International Conference Proceedings 6: 1192–1198. https://doi.org/10.12955/cbup.v6.1314

Tzankova D, Vladimirova S, Peikova L, Georgieva M (2019a) Synthesis and preliminary antioxidant activity evaluation of new pyrrole based aryl hydrazones. Bulgarian Chemical Communications 51(Special Issue A). [in press]

Tzankova D, Vladimirova S, Aluani D, Yordanov Y, Peikova L, Georgieva M (2019b) Synthesis, in vitro safety and antioxidant activity of new pyrrole hydrazones. Acta Pharmaceutica. [in press]

Yurttaş L, Özkay Y, Kaplancıklı ZA, Tunalı Y, Karaca H (2013) Synthesis and antimicrobial activity of some new hydrazone-bridged thiazole-pyrrole derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry 28(4): 830–835. https://doi.org/10.3109/14756366.2012.688043

Vladimirova S, Georgieva M, Zlatkov A (2016) Application of a gradient UHPLC method for determination of chemical and physiological stability of pyrrole-containing ester derivative. Pharmacology, Drug Development & Therapeutics 1: 1–5. https://doi.org/10.15761/PDDT.1000103

Ragavendran JV, Sriram D, Patel SK, Reddy IV, Bharathwajan N, Stables J, Yogeeswari P (2007) Design and synthesis of anticonvulsants from a combined phthalimide–GABA–anilide and hydrazone pharmacophore. European Journal of Medicinal Chemistry 42(2): 146–151. https://doi.org/10.1016/j.ejmech.2006.08.010

Xia Y, Fan CD, Zhao BX, Zhao J, Shin DS, Miao JY (2008) Synthesis and structure–activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells. European Journal of Medicinal Chemistry 43(11): 2347–2353. https://doi.org/10.1016/j.ejmech.2008.01.021