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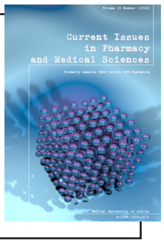

Oxidative coupling reactions: a visual method for determining anthranilic acid derivatives

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ABSTRACT

The anthranilic acid derivative, anthranilic hydrazide, is a very important compound in medicine, although it was recently developed to reduce the side effects of anthranilic acid. Since anthranilic acid derivative hydrazide is a newly produced product, there are few methods for estimating this significant component. Consequently, a simple, inexpensive, and rapid spectrophotometric approach has been developed to determine this chemical in various samples. The two-step method consists of oxidizing the compound with potassium periodate in an acidic medium. A red compound gradually forms after coupling with the 2-amino-4-chloro phenol reagent. Then, the concentration of the compound steadily increases in the second step. The method followed Beer's law at concentrations ranging from 1 to 32.5 μg/mL with a Sandall's value of 0.0245 μg^{-2} cm^{-1} and a molar absorptivity of of 0.899×10^4 L mol^{-1} cm^{-1}. This method was successful in determining the amount of anthranilic acid derivative hydrazide in several samples.

INTRODUCTION

Anthranilic acid (Figure 1), a colorless crystalline solid, is an aromatic amino acid and a precursor to the antibiotic gentamicin and the dye anthranilate. It is used as a fertilizer and an intermediate in the synthesis of many drugs, agricultural chemicals, and dyes. Also known as 2-aminobenzoic acid, anthranilic acid is a natural compound with many medical applications, including the production of antidepressant drugs, such as imipramine, and the treatment of migraines, where it is believed to inhibit the release of neurotransmitters that contribute to headache pain. It is also used as an anticonvulsant, a topical infection treatment, and an antiviral agent, where it is effective against certain viruses, including the herpes simplex virus [3]. Anthranilic acid is highly soluble in chloroform, pyridine, ethanol, and diethyl ether and sparingly soluble in benzene and trifluoroacetic acid [4]. The molecule features a benzene ring connected to two functional groups, a carboxyl group and an amine group, which are positioned ortho to each other. This structural arrangement gives the amino acid a viscous nature, resulting in a white solid in its pure form, though commercial samples often appear green. It plays a significant role in producing azo dyes. Additionally, esters of this amino acid are used in the fragrance industry and in soy sauce production as bacteriostatic agents. Furthermore, it is involved in the manufacture of benzene [5,6].

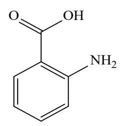

Figure 1. Chemical structure of anthranilic acid

Anthranilic acid (AncH) is present in many natural materials, including nucleic acids and proteins [7], where it is part of the composition of nucleotides; natural pigments, which play an important role in the colors found in plants and animals; and pharmaceutical materials, which it helps determine the chemical properties of. It interacts with a number of compounds to form complex nuclei [8-10]. Using 2-aminobenzoic acid as a core structure to synthesize various compounds exhibits unique pentagonal characteristics that could have biological significance in the future. Following their initial characterization, anthranilic hydrazides (Figure 2) are gaining recognition as compounds of interest. They are valuable starting materials for synthesizing important derivatives due to their unique five-membered non-extrinsic ring structure.

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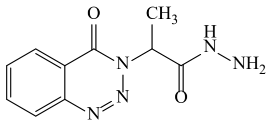

2-(4-oxobenzo[*d*][1,2,3]triazin-3(4*H*)-yl)propanehydrazide

Figure 2: Chemical structure of the hydrazide derivative of anthranilic acid (AncH) [11]

The equation below demonstrates the preparation of AncH from anthanilic acid, highlighting the chemical transformations involved in the process [12].

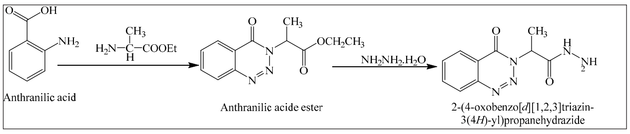

Figure 3: Preparation of anthranilic acid hydrazide

In this context, a hydrazide derivative was successfully synthesized by reducing the anthranilic acid ester using hydrazine hydroxide in ethanol. The anthranilic acid ester was prepared by dissolving it in an aqueous solution of hydrazine. The resulting precipitate was collected and recrystallized, yielding green crystals with a melting point of 180°C. This method paves the way for further exploration of the potential applications and biological significance of these derivatives in future research (Figures 4, 5, and 6; Table 1) [13-18].

Table 1. ^{1}HNMR and ^{13}CNMR spectral data of anthranilic acid hydrazide (AncH)

Structure	^{1}HNMR (400 MHz, DMSOd6), δ (ppm)	^{13}CNMR (100 MHz, DMSOd6), δ (ppm)
AncH	1.73(3H,d,CH_3), 4.34(1H,q,CH), 5.51 (2H, s, NH_2), 7.88-8.23 (4H, m, aromatic CH), 9.42 (1H, s, NH)	17.01 (CH_3), 57.30 (C–N), 119.21-143.89 (aromatic C), 155.26-170.04 (C=O)

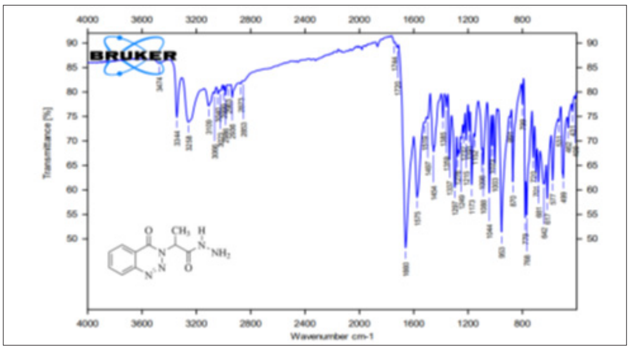

Figure 4. FTIR spectrum of anthranilic acid hydrazide (AncH)

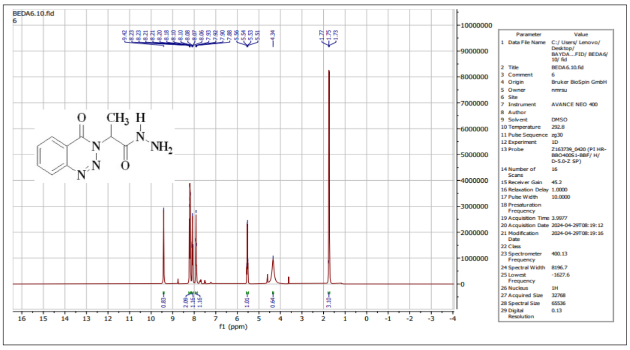

Figure 5. ^{1}H-NMR of AncH

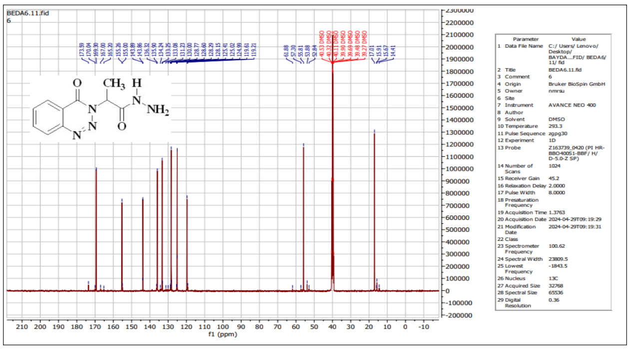

Figure 6. ^{13}C-NMR of AncH

To determine one of the most significant anthranilic acid derivative drugs, anthranilic acid derivative hydrazide, this work provided an easy, accurate, and straightforward spectrophotometric approach. This approach has effectively been used to determine AncH in various materials. The approach is based on oxidizing AncH with potassium periodate in an acidic medium and then coupling the resulting product of the oxidation process with 2-amino-4-chlorophenol.

METHODS

Equipment

A double-beam UV-visible spectrophotometer (JASCO V-630) equipped with 1 cm plastic cuvettes was used for all absorbance measurements of anthranilic acid hydrazide (AncH). The pH of the solutions was measured using a HANA pH meter.

Chemical solutions

A standard solution of anthranilic acid hydrazide (AncH) (100 µg/mL) was prepared according to the reported procedure [16] by dissolving 0.01 g of the compound in ethanol and diluting to volume with distilled water in a 100 mL volumetric flask.

A 0.1% potassium periodate solution (BDH) was prepared by dissolving 0.1 g of the reagent in distilled water and diluting to volume in a 100 mL volumetric flask.

A 1 M hydrochloric acid solution was prepared by appropriate dilution of concentrated hydrochloric acid with distilled water in a 100 mL volumetric flask.

A 0.1% solution of the coupling reagent was prepared by dissolving 0.1 g of 2-amino-4-chlorophenol (98%, Fluka) in ethanol and diluting to volume with distilled water in a 100 mL volumetric flask.

RESULTS AND DISCUSSION

Because of its biological, medical, and agricultural importance, anthranilic acid hydrazide (AncH) was selected for investigation in the present study. The proposed method was developed and applied for the determination of AncH in various samples. To establish the optimum conditions for the analytical procedure, the effects of several experimental variables influencing the measurement process were systematically investigated.

Effect of acid

An acidic environment is the most effective medium for the redox reaction between AncH and potassium periodate. To optimize this process, we examined various acids, including acetic acid, sulfuric acid, and hydrochloric acid, in volumes ranging from 0.5 to 4 mL. Figure 7 shows that 1.0 mL of hydrochloric acid is the optimal amount.

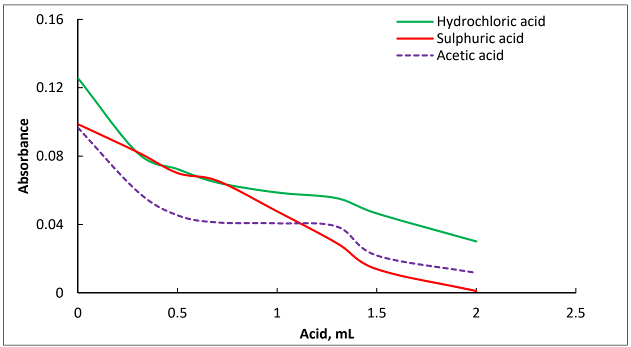

Figure 7. Effect of different acids on the absorbance

Effect of potassium periodate

Ten-milliliter volumetric flasks containing 100 µg of AncH were filled with varying quantities of the oxidizing agent, potassium periodate, ranging from 0.1 to 3.0 milliliters. Each flask was then filled to the brim with distilled water, 1 mL of the ideal coupling reagent concentration, and the previously investigated acid concentration. The absorbance was then measured at a wavelength of 508 nm in comparison to a blank solution. According to this study, 1.5 mL of potassium periodate produced the optimal absorbance values (Figure 8); therefore, this volume was chosen for further investigation.

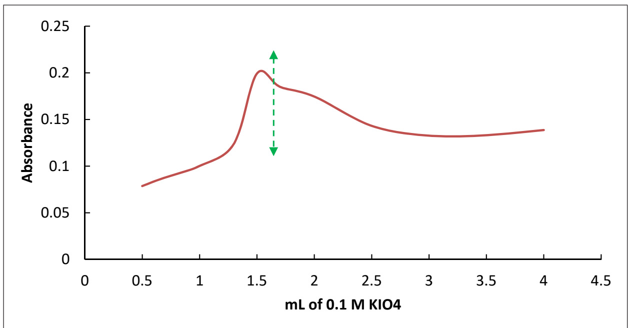

Figure 8. Effect of potassium periodate volume on the absorbance

Oxidation Time

The oxidation process in the examined reaction takes 3 to 5 minutes to complete. To ensure the reaction was complete, a five-minute waiting period was implemented, which was verified in subsequent trials.

Effect of 2-amino-4-chlorophenol (2C4N)

Volumetric flasks containing 100 µg of AncH and 1.5 mL of 0.1% potassium periodate were filled with various volumes of 0.1% 2C4N ranging from 1 to 5 mL to determine the optimal amount of 2C4N required. According to the data, the optimum absorbance values were obtained by adding 2 mL of 0.1% 2C4N; therefore, this volume was chosen for further research.

Effect of base

To optimize the type and quantity of base on the absorbance of the colored product, various bases, including 1 M sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate, and ammonium hydroxide, were examined at different volumes ranging from 0.1 to 3 mL. Figure 4 shows that 1.0 mL of sodium hydroxide is the optimal amount.

Study of the addition of various types of surfactants

Cetylpyridinium chloride (positive surfactant), sodium dodecyl sulfate (negative surfactant), and Triton X-100 (neutral surfactant) were among the surface-active substances evaluated in this investigation to determine AncH. However, the results showed that none of these surfactants produced satisfactory results or had a discernible impact on the chosen wavelength or the absorption intensity of the generated complex. Consequently, this part of the research was disregarded.

Effect of order of addition

The order in which the components were added during the reaction to estimate the anthranilic acid derivative affected the absorption intensity of the resultant complex. Table 2 shows the results of evaluating various sequences to determine the best order for adding the reaction components.

Table 2. Sequence of addition for the reaction components

Sequence	Reaction components	Absorbance
I	AncH + OX + OH + 2C4N	0.4792
II	AncH + OH + OX + 2C4N	0.5148
III	AncH + 2C4N + OH + OX	0.2111
IV	AncH + OX + 2C4N + OH	0.3231
V	AncH + OH + 2C4N + OX	0.1929

AncH = Anthranilic acid hydrazide, OH = NaOH, OX = potassium periodate, 2C4N = 2-amino-4-chlorophenol

The reaction under study stabilizes after about five minutes and remains stable for approximately one hour. These findings were discovered while investigating how time affects the stability and formation of the final colored chemical product. To assess the stability of the colored product, a five-minute waiting period was established. This timing was confirmed in later studies.

Calibration curve

A series of clean, dry 10 mL volumetric flasks containing different concentrations of anthranilic acid derivative within the range of 1 to 32.5 µg were used to study the standard curve of AncH infusions. Then, 1.5 mL of a 0.1% solution of the oxidizing agent KIO_4 was added to the flasks. After five minutes, 2 mL of a 0.1% solution of the 2,4-dinitrophenylhydrazine (2,4-DNPH) coupling agent was added. Figure 9 displays the AncH standard curve using this approach.

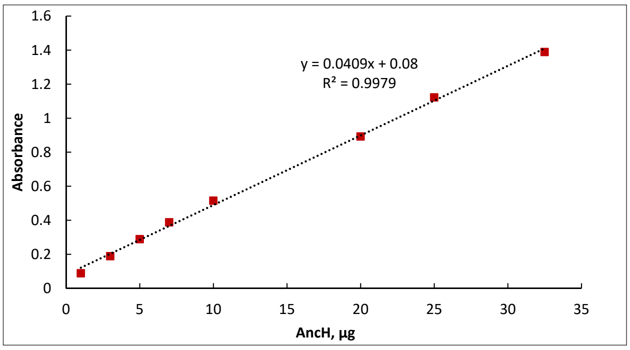

Figure 9. Calibration curve of anthranilic acid hydrazide (AncH)

As shown in Figure 9, the proposed method obeyed Beer's law over the concentration range of 1–32.5 μg/mL. The molar absorptivity was found to be 0.899×10^4 L mol^{-1} cm^{-1}, while Sandell's sensitivity was 0.0245 μg cm^{-2}. The method was successfully applied to the determination of anthranilic acid hydrazide (AncH) in various samples and pharmaceutical preparations containing anthranilic acid derivatives.

Following optimization of the experimental conditions, the absorption spectrum of the colored product was recorded at 508 nm. The final absorption spectrum of AncH is shown in Figure 10 [19]. The proposed method was successfully applied to the analysis of different sample matrices.

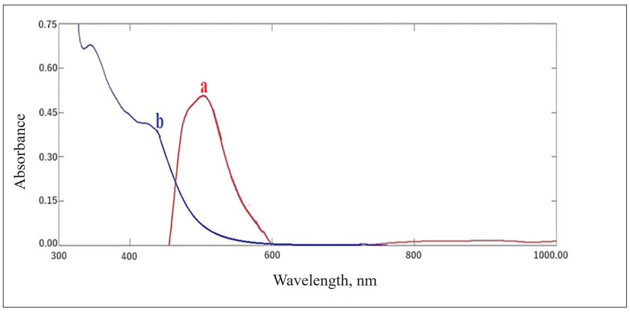

Figure 10. Final spectrum of 1,700 μg of AncH measured against: (a) blank and (b) blank measured against distilled water

Accuracy and precision

To evaluate the accuracy and precision of the proposed method, three concentration levels of anthranilic acid hydrazide (AncH) were analyzed. The corresponding recovery values and relative standard deviations (RSDs) are presented in Table 3.

Table 3. Accuracy and precision of the current method

AncH taken (μg)	Recovery* (%)	AncH measured (μg)	R.E.* (%)	RSD* (%)
25	99.48	23.70	−0.52	±0.311
100	100.07	100.07	+0.07	±0.283
200	101.24	202.48	+1.24	±0.190

* - average of 5 determination

Table 3 shows that the proposed method provides a high level of reliability, confirming its suitability for the intended analytical applications.

Nature of the reaction

To determine the reaction ratio between the 2,4-dinitrophenylhydrazine (2C4N) coupling agent and the anthranilic acid derivative, a continuous variables approach using Job's method was employed. This methodology effectively elucidates the reaction dynamics, as depicted in Figure 6. It provides significant insight into the interaction between the two compounds. Figure 11 shows that the reaction ratio of AncH to 2C4N is 1:1, as confirmed by the experimental results.

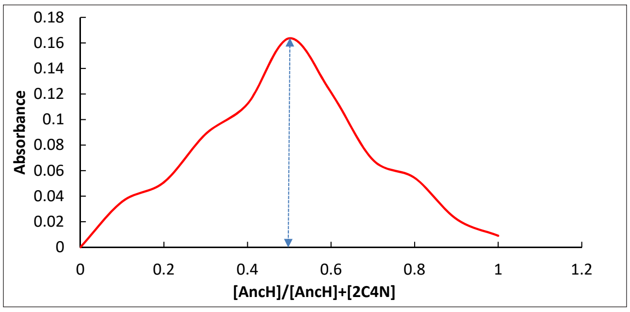

Figure 11. Job's Plot for AncH

Effect of interferences

The effects of various potentially interfering substances were investigated to evaluate the selectivity of the proposed method. These substances were tested in the presence of 100 μg of anthranilic acid hydrazide (AncH). The results obtained are summarized in Table 4.

Table 4. Effect of interfering substances on the determination of anthranilic acid hydrazide

Interferences	Recovery (%) of 100 μg AncH/μg of interference added		
	300	500	1000
Ammonium chloride	98.43	97.69	97.74
Boric acid	98.26	97.67	98.41
Calcium sulphate	99.45	97.62	99.93
Glucose	98.64	98.32	98.18
Glycerol	99.62	99.81	99.23
Hydrazine sulphate	101.23	102.02	103.44
Hydroxylamine-HCl	96.91	97.98	96.97
Lactose	98.67	98.82	98.62
Magnesium sulphate	97.95	98.89	99.58
Menthol	97.90	97.87	97.94
Phenyl hydrazine	96.98	96.34	96.56
Sodium chloride	99.69	98.62	98.58
Sodium persulphate	97.96	99.97	99.95
Sodium sulphate	99.95	98.99	98.92
Starch	99.94	99.96	99.94
Sucrose	99.96	99.98	99.91
Thoiurea	96.99	99.94	99.90
Urea	96.91	99.99	99.98

Application of the method

The proposed method was successfully applied to the determination of anthranilic acid hydrazide (AncH) in various samples. As shown in Table 5, satisfactory recovery values were obtained, demonstrating the applicability and reliability of the method.

Table 5. Application of the proposed method to environmental and pure samples

AncH (μg)	Recovery (%) of AncH*									
	River water from Tigris in Mosul city, Iraq (ml)		Well water from Al-Qaser village, Mosul, Iraq (ml)		Sea water Turkey (ml)		Tap water (ml)		Anthranilic acid derivative pure	
	2	4	2	4	2	4	2	4	100	300
100	97.62	97.92	101.12	97.58	100.09	102.09	98.37	98.28	99.14	99.33
300	101.09	102.11	101.03	97.32	102.13	102.13	98.69	98.80	99.04	99.16
500	99.85	99.15	102.11	98.11	101.37	102.42	98.22	99.65	99.18	99.01

* - average of 5 determination

Evaluation of the proposed method

The performance of the proposed method for the determination of anthranilic acid hydrazide (AncH) in various samples was evaluated by statistical comparison with the reference method. Student's t-test and F-test were applied at the 95% confidence level, and the results are presented in Table 6. The calculated t- and F-values were lower than the corresponding tabulated values (t = 2.306 and F = 6.39) at the same confidence level [19], indicating that there was no significant difference between the proposed method and the reference method [20,21]. These results demonstrate that the proposed method is comparable to the reference method in terms of accuracy and precision.

Table 6. Evaluation of 100 μg AncH

Sample	AncH Found, μg*		Reco.*(%) ± RSD*(%)		Value of	
	Present method	Ref. meth#	Present method	Ref. meth#	t^a	F^b
Tap water	99.33	101.03	99.33±0.55	101.03±1.39	1.15	0.79
Anthranilic acid derivative pure	98.67	98.11	98.67±0.68	98.11±0.89	0.679	0.835
Sea water	97.13	96.78	97.13±1.24	96.78±1.51	1.55	1.08
Well water	97.91	97.45	97.91±0.51	97.45±1.15	1.17	0.692

(Saleem et al., 2022), * - average of 5 determination, a - degree of freedom for t-value (n=8), b - degree of freedom for F-value (n=4)

Table 7 lists the analytical variables used in this method..

Table 7. Analytical variables

Analytical variables	Present methods
Reaction	Oxidative coupling
λ_{max} (nm)	508
Reagent	2-amino-4-chloro phenol
Beer's law range (μg·mL^{-1})	1-32.5
Molar absorptivity (L.mol^{-1}·cm^{-1})	0.899×10^4
Sandall's sensitivity (μg^{-2}·cm^{-1})	0.0245
Color's stability (minutes)	> 60
RSD%	±0.190 - ±0.311
Method's application	Various samples

CONCLUSIONS

One important anthranilic acid derivative, anthranilic acid hydrazide, can be identified using a spectroscopic technique. This technique is notable for its sensitivity and ease of use, offering a quick, affordable, and reasonably accurate method. Statistical comparisons and visual parameters support its application for the routine estimation of anthranilic acid derivatives in different samples. Additionally, this process is well-suited for assaying anthranilic acid hydrazide in various samples because it does not require complicated sample preparation or specific reaction conditions.

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