Iraqi National Journal of Chemistry

Journal homepage:
http://iqnjc.com/Default.aspx

Simultaneous Spectrophotometric Determination of Mesalasine and Isoniazid Using H-Point Standard Addition Method via Schiff's Base Formation Reaction

Theia'a N. Al-Sabha* and Nagham N. Habeb
Chemistry Department, College of Education, Mosul University, Mosul, Iraq

Abstract

H-point standard addition method (HPSAM) has been applied to the simultaneous spectrophotometric determination of mesalasine (*5-ASA*) and isoniazid (*INH*) drugs in binary mixtures. The method is based on the Schiff's base formation of drugs with 1,2-naphoquinone sulphonate (NQS) as a chromogenic reagent in the presence of micellar cityltrimethyl ammonium bromide (CTAB) and sodium carbonate. The results showed that simultaneous determinations could be performed with the ratios 0.4:1.4 and 1.6:0.2 measured at pair of wavelengths (438 and 521 nm) with recovery % range 94.5-105 % and precision better than 2.95 %

Keywords: Isoniazid; Mesalasine; Simultaneous determination; Spectrophotometry; HPSAM

تطبيق طريقة الإضافة القياسية-النقطة المشتركة في التقدير الطيفي الآني للأيزونايزايد والميزالازين باستخدام تفاعل تكوين قاعدة شيف

ضيـاء نجم الصبحة ونغم ناظم حبيب

قسم الكيمياء- كلية التربية للعلوم الصرفة-جامعة الموصل-العراق

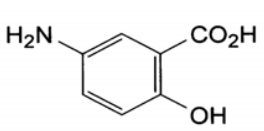
الخلاصة

تم تطبيق طريقة الإضافة القياسية – النقطة المشتركة في التقدير الآني لدوائي الأيزونايزايد والميزالازين في الخليط الثنائي. تعتمد الطريقة على تكوين معقدات شيف لتلك الأدوية بواسطة تفاعلهما مع الكاشف الكروموجيني ٢،١-نفثوكوينون سلفونات بوجود عامل الشد السطحي سيتايل ثلاثي مثيل بروميد الأمونيوم وكاربونات الصوديوم. لقد أثبتت النتائج بإمكانية تقدير الدوائين أعلاه بنسبة ٠.٤:١.٤ و ١.٦:٠.٢ على التوالي والمقاسة عند زوج الأطوال الموجية ٤٣٨ و٥٢١ نانوميتر. تراوحت نسبة الاسترجاعية بين ٩٤.٥% و ١٠٥% وبتوافقية أفضل من ٢.٩٥%.

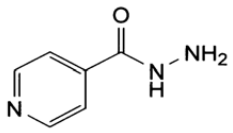
الكلمات المفتاحية: ميزالازين، ايزونايزايد، التقدير الآني، إضافة قياسية-نقطة مشتركة.

Introduction

Mesalasine is chemically known as 5-amino-2-hydroxy benzoic acid; (*5-ASA*), (I), also known as mesalamine, is an anti inflammatory drug used to treat inflammation of the digestive tract (crohn's disease) and mild to moderate ulcerative colitis. It is a bowl specific amino salicylate drug that is metabolized in the gut and has its predominant actions there, thereby having fewer systemic side effects [1].
Isoniazid (*INH*; isonicotinoylhydrazine) (I), is the most potent and selective tuberculostatic antibacterial agent in the therapy of tuberculosis [2]. It inhibits the growth of tubercle bacillus in vitro in concentration less than 1 μg ml-1. It is also used as a prophylactic agent for persons constantly exposed to tubercular patients [3].



(I)



(II)

Several spectrophotometric methods depending on using different reagents have been reported for the determination of the intended drugs. Resorcinol [4], chromotropic acid and phloroglucinol [5], tetracyanoethylene (TCNE) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [6] are used for the determination of *5-ASA*. N-Bromosuccinimde [7], 4,5-dihydroxy-1,3-benzenedisulfonic acid in the presence of sodium metaperiodate [8], diazotized 4,4-methylene-bis-m-nitroaniline [9], 4,4'-sulphonyldianiline [10], and 1,2-naphthoquinone-4-sulfonic acid [11] are used for the determination of isoniazid. However; these methods are depended upon the determination each of *INH* and *5-ASA* alone and in their pharmaceutical formulations. A colorimetric reaction for simultaneous determination of *INH* and *d*-cycloserine was described using sodium pentacyanoammineferroate reagent [12].
Vireodt's method (simultaneous equations) was described for simultaneous determination of *INH* and *5-ASA* [13] or *5-ASA* and prednisolone [14] in binary mixtures. Partial least squares regression was used for the simultaneous quantification of *INH* and rifampicin by visible spectrophotometry using a simple derivatization reaction in the presence of neocuproine, copper (II) [15]. A method for simultaneous determination of *INH* and *5-ASA* by capillary electrophoresis using chemiluminescence detection was described [16]. Other analytical methods have been reported for simultaneous determination of *INH* or *5-ASA* with other drugs in binary mixtures such as chromatography[17,18].
It was found *INH-5-ASA* combination commonly used in therapy was clastogenic. From this observation it may be concluded that *INH* and *5-ASA* act synergistically in producing chromosomal aberrations [19]. However; simultaneous estimation methods have not been so far reported for the *INH* and *5-ASA* spectrophotometrically. In this work, a sensitive, selective, accurate and inexpensive procedure for simultaneous spectrophotometric determination of *5-ASA* and *INH* in binary mixture by HPSAM, using NQS as chromogenic reagent, has been proposed.

Theoretical Background

Falco *et al.* proposed a modification of MOSA, the H-point standard addition method (HPSAM), which makes it possible to determine the concentration of analyte in the presence of a direct interferent and even the concentration of interferent can be determined. The basis of the method for spectrophotometric determination of binary

mixtures with extensively overlapped spectra and in the presence of proportional errors was established [20,21].

The HPSAM as applied to equilibrium and spectrophotometric data allows the determination of two species X and Y in a mixture, even if their spectra are completely overlapped or only the analyte concentration free of bias error when the spectrum of the sample matrix is known. The determination of the concentration of X by the HPSAM under these conditions entails selecting two wavelengths λ_1 and λ_2 lying on each side of the absorption maximum of Y and at the same distance if the peak is regularly shaped such that the absorbances of this latter component are the same at both. Then, known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two aforesaid wavelengths. The two straight lines thus obtained intersect at the so-called "H-point" $(-C_H, A_H)$, where $-C_H$ $(=-C_x)$ is the unknown concentration of X and A_H $(=A_y)$ is the analytical signal of Y in the former case and represents the constant bias error of the sample in the latter [22].

In this work HPSAM has been applied for the determination of 5-ASA and INH in binary mixtures as pure forms and their pharmaceutical formulations.

EXPERIMENTAL SECTION

Apparatus

Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**,** Shimadzu UV-1650 PC, UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell**,** All calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

A standard solutions of 50 µg ml^{-1} 5-ASA of and INH were prepared separately in 100 ml volumetric flask by dissolving 0.005 g in 2.0 ml of ethanol and diluting to the mark with distilled water. They were stored in dark and were found to be stable for at least 4 weeks. 5×10^{-3} M of NQS reagent was prepared by dissolving 0.065 g in distilled water in a 50 ml volumetric flask. 0.1 M sodium bicarbonate was prepared in a 500 ml volumetric flask. 0.1 % of CTAB was prepared in warm distilled water. All reagents were of analytical grade (BDH , Fluka and Molekula companies).

Procedure

Individual calibration

Standard solutions of 0.0-4.8 and 0.4-4.0 µgml^{-1} for 5-ASA and INH were added into two sets of 25-ml volumetric flasks respectively, followed by addition of 0.2 ml NQS reagent solution, 2.0 ml sodium bicarbonate and 1.5 ml CTAB individually. The solution was made up to the mark with distilled water, and was left for 10 min at room temperature. A portion of the solution was transferred into a 1cm silica cell to measure the absorbance at 505 and 480 nm against their respective reagent blank for 5-ASA and INH, respectively .

H-point standard addition analysis

Synthetic samples containing different concentrations of *5-ASA* and *INH* standard solutions were prepared and added into 25-ml volumetric flasks, followed by addition of standard addition of *5-ASA* (up to 2.0 µg ml^{-1}), 0.2 ml NQS, 2.0 ml sodium bicarbonate and 1.5 ml CTAB. The volume was made up to the mark with distilled water and left for 10 min at room temperature. Simultaneous spectrophotometric

determination of *5-ASA* and *INH*, in their binary mixture, was made with HPSAM at 438 and 521 nm. *5-ASA* or *INH* can be determined simultaneously in the range 0.4-1.6 and 0.2-1.4 μg ml^{-1} respectively. The procedure was repeated for some synthetic mixtures to show applicability of the method.

Results and Discussion

5-ASA and *INH* were reacted with NQS reagent and gave an orange color with λ_{max} at 505 nm for *5-ASA* and red color with λ_{max} at 480 nm for *INH* Schiff base in presence of CTAB (Figure1). However; it was found the absorption maxima of the drugs are nearly identical and it is difficult to determine them by classical technique. Therefore, use a chemometric method is necessary to solve this problem.

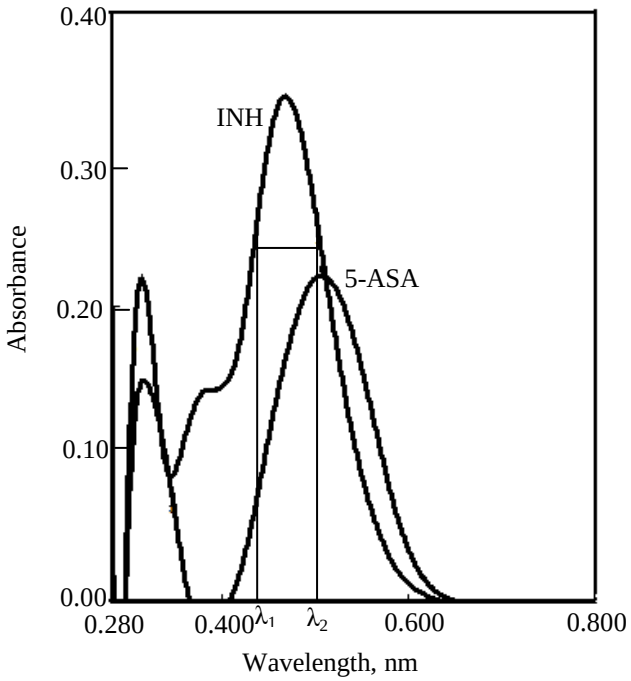


Figure 1. Absorption spectra of NQS product with 4 μg ml^{-1} INH and 5-ASA at the optimum conditions.

Effect of Variables

The parameters including effect of pH, type of base, concentration of NQS, surfactant and temperature were optimized by setting all parameters constant and optimizing one at a time.

Effect of pH

The effect of final pH in the range between 2.0 and 11.0 in the final volume on the absorption spectra for solutions containing *INH* and *5-ASA* (4 μg ml^{-1}) was studied by addition of 0.1 M of HCl and NaOH. It was found that the products were formed in basic medium with maximum absorption at pH of 11.7 at 478 and 477 nm for *INH* and *5-ASA* respectively.

Effect of bases

The effect of bases like sodium hydroxide, sodium bicarbonate, sodium carbonate, potassium hydroxide and ammonium hydroxide have been examined To obtain high sensitivity for the products,. However; it was found that sodium carbonate gave more stable color at 477 and 462 nm for *5-ASA* and *INH* respectively. Fig. 2 shows that concentration ranges of 1.0 - 4.0 ml and 1.5 - 4.0 ml of 0.1 M sodium

carbonate with pH range between 9.91-10.15 and 10.08-10.15 yielded maximum absorption for *5-ASA* and *INH* respectively. So, 2.0 ml of sodium bicarbonate was used in subsequent experiments.

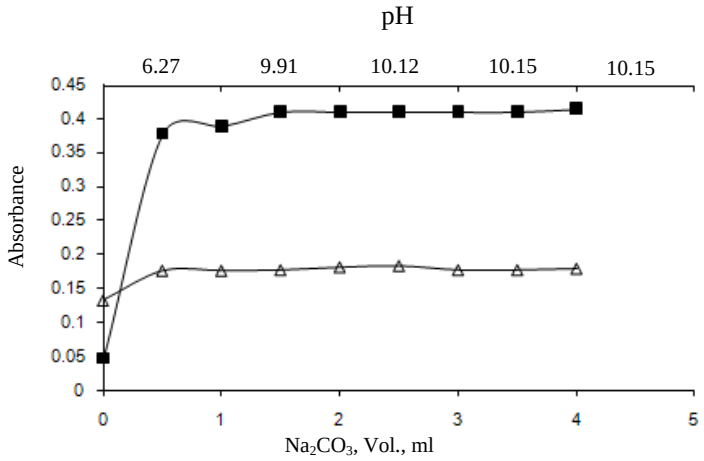


Figure 2. Effect of Na_2CO_3 concentration on the absorption of reaction mixture of 4 μg ml^{-1} for each (Δ) *5-ASA* measured at 477 nm and (■) *INH* measured at 462 nm in the presence of NQS reagent.

Effect of NQS reagent

It was found that the absorbance of both drugs increased by increasing the volume of 5×10^{-3} M NQS concentration up to 0.2 ml and remained constant at higher concentrations up to 1.0 ml for both drugs (Figure 3), measured at their respective λ_{max} cited in Table 1, after which the absorbance decreased. Therefore, 0.2 ml of 5×10^{-3} M NQS was selected as optimum concentration.

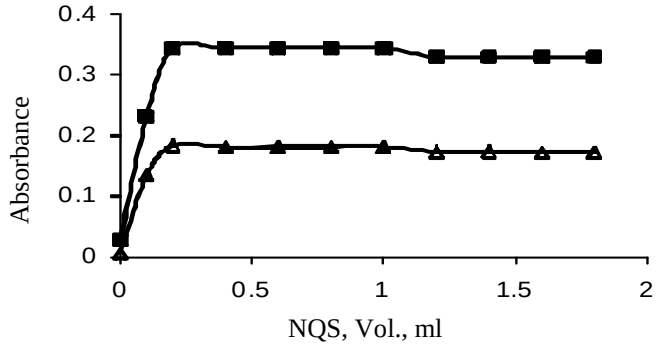


Figure ٣. Effect of NQS reagent concentration on the absorption of reaction mixture of 4 μg ml^{-1} for each (Δ) *5-ASA* measured at 477 nm and (■) *INH* measured at 462 nm in the presence of Na_2CO_3 solution.

Effect of surfactants

The effect of cetyltrimethyl ammonium bromide (CTAB), Tween-80 (TW-80) and TritonX-100 (TX-100) of 0.1 % concentration on the absorption spectra of products have been investigated. It was found that cationic surfactant CTAB shift the maximum absorption to longer wavelength (18 nm for *INH* and 30 nm for *5-ASA*) (Table 1) and increased the absorbance of *5-ASA* product, the anionic SDS, nonionic TX-100 and TW-80 surfactants showed increasing in absorbance of *5-ASA* but

shifting to the shorter wavelength. However; CTAB was most effective, and gave stable color and reproducible. The absorbance increases with CTAB concentration up to 1.0 ml and 1.5 ml which were remain constant up to 2.0 ml and 3.0 ml for *5-ASA* and *INH* respectively, (Fig. 4). However; 1.5 ml of 0.1% CTAB was selected for next investigation.

Table 1. Effect of surfactants on the absorption of 4 µg ml^{-1} for each *5-ASA* and *INH* products.

Surfactant	*INH*		*5-ASA*	
	λ_{max} (nm)	Abs.	λ_{max} (nm)	Abs.
Without	462	0.364	477	0.182
CTAB	480	0.368	507	0.272
SDS	469	0.365	467	0.296
TX-100	463	0.359	466	0.304
TW-80	464	0.356	466	0.294

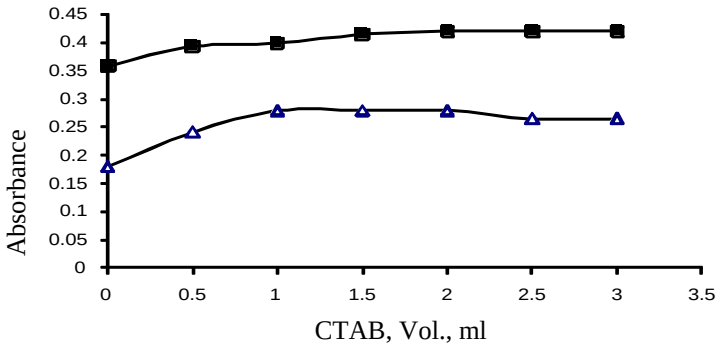


Figure 4. Effect of CTAB concentration on the absorption of reaction mixture of 4 µg ml^{-1} for each (Δ) *5-ASA* and (■) INH in the presence of NQS and Na_2CO_3 solution.

Effect of temperature and developing time

The effect of room temperature (25°C) and 40°C on the rate of reaction for both drugs was studied. The results indicated that products were formed after addition of reagents immediately at room temperature and reached its maximum absorbance after 5.0 min at 507 and 480 nm for *5-ASA* and *INH* respectively and remains stable for more than 1h, but a decrease in absorbance with time was noticed at 40°C. Therefore, 10 min at 25°C was used in this work.

Quantification

The Beer's law limits and molar absorptivity values were evaluated (Table 2). The corresponding correlation coefficient for the drugs determined by the proposed method represents excellent linearity. The average recovery % and relative standard deviation (RSD) for the analysis of six replicates of each three different concentrations for drugs indicated that the method is precise and accurate. Limit of quantitation (LOQ) is determined by taking the ratio of standard deviation of the blank with respect to water and the slope of calibration curve multiplied by a factor of

10. LOQ is approximately 3.3 times greater than LOD. Naturally, the LOQ slightly crosses the lower but LOD is well below the lower limit of Beer's law range.

Table 2. Optical characteristics and statistical data for the proposed method.

Parameter	***INH***	***5-ASA***
Beer's law limits ($\mu g\ ml^{-1}$)	0.4-4.0	0.0-4.8
Molar absorptivity ($l.mol^{-1}.\ cm^{-1}$)	1.63×10^{4}	9.00×10^{3}
LOD ($\mu g\ ml^{-1}$)	0.0206	0.0687
LOQ ($\mu g\ ml^{-1}$)	0.0418	0.1394
Average recovery (%)**	97..9	101.3
Correlation coefficient	0.9971	0.9989
Regression equation (Y)*		
Slope, *a*	0.1229	0.0588
Intercept, *b*	0.0231	0.0027
RSD**	≤ 1.076	≤ 2.163

*$Y = a\ X + b$, where X is the concentration of analyte in $\mu g\ ml^{-1}$.
** Average of six determinations.

Simultaneous spectrophotometric determination of 5-ASA and *INH* using H-point standard addition method

HPSAM has been applied for simultaneous determination of *5-ASA* and *INH* in binary mixtures according to the optimum conditions obtained above and cited in (Table 3).

Table 3. Compromised reaction conditions for determination of *5-ASA* and *INH* drugs

Reagent	**Conc.**	**Vol., (ml)**	**Temp. (°C)**	**Time (min)**
NQS	5×10^{-3} M	0.2	25	10
Na_2CO_3	0.1 M	2.0		
CTAB	0.1 %	1.5		

Wavelength selection

To select the appropriate wavelength pair for using HPSAM the following principles should be applied [23-25].

1. The analyte signals must be linear with concentration and the interferant signal must be equal and remaining unchanged by changing the analyte concentration at the selected wavelengths.
2. The analytical signal obtained from a mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components.
3. The difference in the slopes of the two straight lines measured at two selected wavelengths (λ_1 and λ_2) must be as large as possible in order to get good accuracy and sensitivity.

In the proposed system, *5-ASA* and *INH* drugs could be considered as the analyte and interfering drug, respectively. At selected wavelengths of λ_1= 438 nm and λ_2 = 521 nm (Fig.1), in this case there were several pairs of wavelengths. The

absorbances of *5-ASA*-NQS product found to increase linearly with the concentration of *5-ASA* drug, whereas, the absorbances of *INH*-NQS product remained equal, even with increasing analyte concentration.

Standard solutions of two drugs were initially tested to validate the applicability of the chosen wavelengths. Known amounts of *5-ASA* are consecutively added to the mixture containing fixed amounts of *5-ASA* and *INH*. The absorbance is measured after each addition at the two selected wavelengths, and expressed by the following equations.

$$A_{438} = M_{438}C_{5\text{-}ASA} + bo + b \qquad (1)$$

$$A_{521} = M_{521}C_{5\text{-}ASA} + Ao + A' \qquad (2)$$

where, A_{438} and A_{521} are the analytical signals measured at λ_{438} and λ_{521}, respectively. b_0 and A_0 ($b_0 \neq A_0$) are the absorbances of *5-ASA* at A_{438} and A_{521}, respectively. b and A' ($b = A'$) are the absorbances of *INH* at A_{438} and A_{521}, respectively. M_{438} and M_{521} are the slopes of the standard addition calibration lines at 438 nm and 521 nm, respectively, and $C_{5\text{-}ASA}$ is the added *5-ASA* concentration.

The two straight lines obtained intersect at the H-point ($-C_{5\text{-}ASA}$, A_{INH}) (Fig. 5). According to the characteristics of HPSAM at the H-point, C_H is independent of interferent concentration, but A_H is dependent of the interferent.

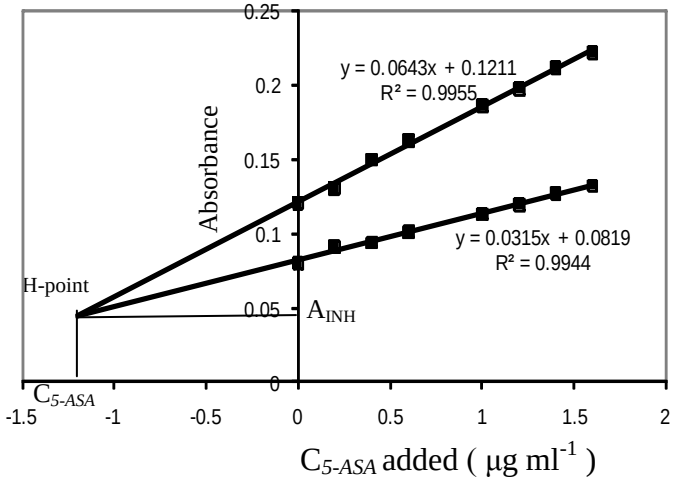


Figure 5. H-point standard addition plot for the simultaneous determination of *5-ASA* and *INH*. Conditions: 0.8 μg ml^{-1} of *INH*, 1.2 μg ml^{-1} of *5-ASA*, NQS = 2 ml of 5×10^{-3}M, time= 10 min. at 25 ^{o}C.

Reproducibility and accuracy of the method

In order to study the reproducibility of the proposed method, three replicate experiments on binary mixtures containing fixed amounts of three different concentrations for *5-ASA* and *INH* were performed. The concentration of interfering (*INH*) was calculated in each solution by calibration method using standard solutions and the ordinate value of H-point (A_H) which is carried out on a series of samples containing a fixed amount of *5-ASA* with varying amounts of *INH* (Fig. 6,7). A good RSD was obtained for *5-ASA* and *INH* (< 3.0 %) and a recovery of 94.5-104.5 %, was obtained in the samples (Table 4).

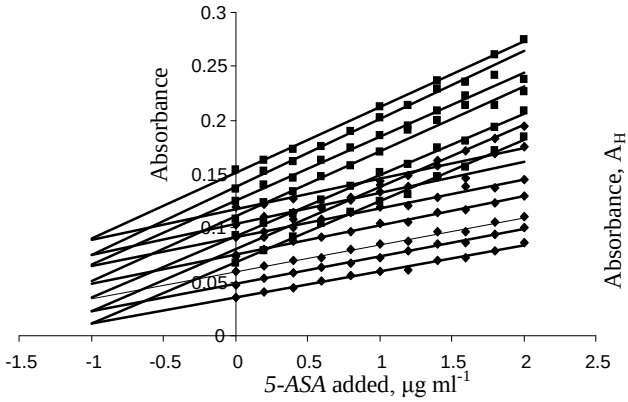


Figure 6. Plots of H-point standard addition method for fixed amount *5-ASA* (1.0 µg ml^{-1}) in the presence of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 µg ml^{-1} of *INH* at (■) 438 nm

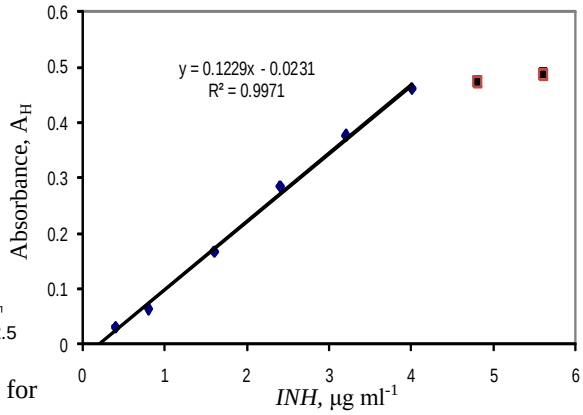


Figure 7. Calibration graph of *INH* in the presence 1.0 µg ml^{-1} *5-ASA*

Table 4. Results of three replicates of three different concentrations for the analysis of *5-ASA* and *INH*

A-C Equation	R^2	Amount taken (µg ml^{-1})		Recovery %		RSD	
		5-ASA	***INH***	***5-ASA***	***INH***	***5-ASA***	***INH***
Y_{438}=0.0556x+0.091 Y_{521}=0.0249x+0.0596	0.9974 0.9983	1.0	0.6	102	99.1		
Y_{438}=0.0566x+0.0901 Y_{521}=0.0254x+0.0594	0.9970 0.9980	1.0	0.6	101	97.9	0.5109	1.076
Y_{438}=0.0561x+0.0905 Y_{521}=0.0257x+0.0595	0.9965 0.9974	1.0	0.6	101	96.8		
Y_{438}=0.0556x+0.0849 Y_{521}=0.0255x+0.0723	0.9939 0.9919	0.4	1.0	104.5	104		
Y_{438}=0.0561x+0.0839 Y_{521}=0.0255x+0.0719	0.9929 0.9960	0.4	1.0	98.0	105	2.955	0.495
Y_{438}=0.0557x+0.0843 Y_{521}=0.0256x+0.0719	0.9928 0.9927	0.4	1.0	102.75	104		
Y_{438}=0.0568x+0.1146 Y_{521}=0.0261x+0.0765	0.9958 0.9963	1.2	0.8	103.3	94.5		
Y_{438}=0.0569x+0.1142 Y_{521}=0.0262x+0.077	0.9962 0.9920	1.2	0.8	100.8	97.1	2.163	0.994
Y_{438}=0.0554x+0.1155 Y_{521}=0.0255x+0.0773	0.9966 0.9933	1.2	0.8	105	96.1		

For simultaneous determination of *5-ASA* and *INH* in a sample, number of synthetic mixtures with different concentration ratios of *5-ASA* and *INH* were analyzed using HPSAM. As cited in Table 5, the accuracy of the results is satisfactory in the concentration ratio of *5-ASA* to *INH* varying from 0.4 : 1.4 and 1.6 : 0.2. Results of the analysis of the different mixtures in the proposed system revealed a dynamic range of 0.4-1.6 and 0.2-1.4 µg ml^{-1} for *5-ASA* and *INH*, respectively.

Table 5. Determination of *5-ASA* and *INH* in some synthetic mixtures

A-C Equation	R^2	Amount taken µg /ml		*5-ASA*		*INH*	
		5-ASA	*I.N.H*	Found (µg ml^{-1})	Recovery %	Found (µg ml^{-1})	Recovery %
Y_{438}=0.022x+0.0368 Y_{521}=0.052x+0.0543	0.9873 0.9979	0.6	0.4	0.58	96.6	0.42	105
Y_{438}=0.0255x+0.0773 Y_{521}=0.0554x+0.1155	0.9933 0.9960	1.2	0.8	1.27	105.8	0.769	96.1
Y_{438}=0.0249x+0.0596 Y_{521}=0.0556x+0.091	0.9983 0.9974	1.0	0.6	1.02	100.2	0.594	99
Y_{438}=0.0257x+0.0898 Y_{521}=0.0564x+0.1092	0.9909 0.9959	0.6	1.2	0.63	105	1.23	102.3
Y_{438}=0.0255x+0.0723 Y_{521}=0.0556x+0.0849	0.9919 0.9939	0.4	1.0	0.41	102.5	1.036	103.6
Y_{438}=0.0235x+0.049 Y_{521}=0.0529x+0.0985	0.9954 0.9987	1.6	0.2	1.68	105	0.191	95.5
Y_{438}=0.0263x+0.0989 Y_{521}=0.0561x+0.1116	0.9818 0.9958	0.4	1.4	0.42	105	1.46	104.2

Effect of exciepients

The influence of the presence of common exciepients on the determination of 1.0 µg ml^{-1} of *5-ASA* and *INH* have been investigated by HPSAM at wavelengths 438 and 521 nm. A placebo blank solutions containing 250, 500 and 1000 µg ml^{-1} for each of starch, acacia, lactose and fructose were prepared.

A synthetic mixtures were separately prepared by adding 1 µg ml^{-1} of pure *5-ASA* and *INH* to the above mentioned placebo blank solutions and the mixtures were shake. *5-ASA* and *INH* were assayed according to the general procedure of H-point standard addition analysis described earlier and the percentage recoveries were computed. Table 6 indicated that no interference effect of exciepients on the determination of *5-ASA* and *INH*.

Table 6. Effect of exciepients

Placebo Blank (µg ml^{-1})	Amount of taken (µg ml^{-1})		Recovery* (%)	
	5-ASA	*INH*	*5-ASA*	*INH*
250	1.0	1.0	97.8	98.5
500	1.0	1.0	96.6	97.0
1000	1.0	1.0	96.0	94.0

* Average for three determinations.

Application of the method

The proposed method has been applied for determination of *5-ASA* and *INH* in their available pharmaceutical formulations as tablets containing 400 mg *5-ASA* and 100 mg *INH*. The results showed in Table 7 that the proposed method was free from interferences in the determination of *5-ASA* and *INH* drugs in the pharmaceutical preparation as tablets.

Table 7. Assay of *5-ASA* and *INH* in pharmaceutical preparations using the proposed method

Pharmaceutical preparation	Amount taken (µg ml^{-1})	Amount Found (µg ml^{-1})	Recovery* (%)	Average recovery (mg)	Certified value (mg)
5-ASA Tablet	0.4	0.39	97.25	386.32	400
	0.8	0.76	95.00		
	1.2	1.17	97.50		
INH Tablet	0.4	0.38	95.25	97.00	100
	0.8	0.81	101.25		
	1.2	1.13	94.50		

* Average for three determinations

Conclusion

HPSAM has been applied for simultaneous determination of 5-ASA and INH drugs. The proposed method based on the reaction of NQS reagent with 5-ASA and INH in micellar medium. The method is simple and offers good selectivity, accuracy and precision. The method is suitable for routine analysis in control laboratories.

References

[1] V. Darak, A. B Karadi, M. D Arshad and S A. raju, Int. J. Pharm. Sci., 2 (4), 31-35, (2011).
[2] S. O Gelone, J. A. Donell, "Anti Infectines", Chapter 90, in Renimgton, The Science and practice of pharmacy, Gerbinoo, P. P. (Directore) 21ist ed., Lippincott Williams and wilkins, Baltimore, Maryland, U. S. A., 2005, p. 1663.
[3] G. R. Chatwal, "Anititubercular and antileprosy drugs", chapter 25, In "Synthetic Drugs". 2nd ed. (Arora M, ed.) Himalya Publishing House, Delhi, 1990, p. 374.
[4] V. Madhavi, V. Panchakshari, T.N. Prathyusha and C.B. Sekaran, Inter. j. pharm. sci. review and research, 105-109,(2011).
[5] V. Darak, A. B. Karadi, Md. Arshad and S. A. Raju, Asian J. Res. Chem., 4, 968-970, (2011).
[6] T. N. Al-Sabha and O. A Altaee, J.Edu & Sci., 21, 49- 62, (2008).
[7] B. N Barsoum, M. S Kamal and M. M. Diab, Res. J. Agr. Bio. Sci., 4, 471- 484, (2008).
[8] B. G. Gowda, M. B Melwanki, J. Seetharamappa and K. C. S. Murthy, Anal. Sci., 18, 839-841, (2002).

[9] G. K. Naidu, K. Suvardhan , K. S. Kumar, D. Rekha, B. S. Sastry and P. Chiranjeevi, J. Anal. Chem., 60, 822-827 (2005).
[10] P. Nagaraja, K. Sunitha, R. Vasantha and H Yaajanthir, Turk. J. Chem., 26, 743-750 (2002).
[11] S. Vijayraj et al., The Experiment, 17, 1205-1212, (2013).
[12] V. Scardi and V. Bonavita, Clin. Chim. Acta, 4, 161–164, (1959).
[13] S. Vijayraj et al., The Experiment, 17, 1205-1212, (2013).
[14] S. Jain, N. Jain, H. Khambete and A. Rawa, Inter. J. Pharm. Sci. Res., 3, 3707-3711, (2012).
[15] S. Stets, T. M. Tavares, P. G. P. Zamora, C. A. Pessoa and N. Nagata, J. Braz. Chem. Soc., 24, 1198-1205, (2013).
[16] X. Zhang, Y. Xuan, A. Sun, Y. Lv and X. Hou, Luminescence, 24, 243-249, (2009).
[17] D. Iriminescu, M. C. Dobrin1, G. Hancu, E. Mircia, H. Kelemen, A. Rusu and M. Tilinca, Seria Ştiinţele Vieţii, 26, 353-357, (2016).
[18] G. Palumbo, G. Carlucci, P. Mazzeo and D. Fanini, J. Pharm. Biomed. Anal., 14,175-80, (1996).
[19] M. Jaju, M. Jaju and YR. Ahuja, Hum Genet.,56,375-7, (1981).
[20] F. B. Reig and P. C. Falco, Analyst,133, 1011-1016, (1988).
[21] P. C. Falcó, F. B. Reig and A. M. Benet, Fresnius J. Anal. Chem., 338, 16-21, (1990).
[22] F. B. Reig, P. C. Falco, A. S. Cabeza, R. H. Hernandez, and C. M. Legua, Anal. Chem., 63, 2424-2429, (1991).
[23] P. C. Falcó, F. B. Reig, R. H. Hernández and A. S. Cabeza, Anal. Chim. Acta, 257, 89, (1992).
[24] E. Shams, H. Abdollahi, M. Yekehtaz, R. Hajian, Talanta, 63, 359, (2004).
[25] A. A. Ensafi and R. Hajian, Anal. Chim. Acta, 580, 236-243, (2006).