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Determination of nicotinamide by stopped-flow injection method in pharmaceutical formulations

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KEYWORDS

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Abstract A simple stopped-flow injection system with spectrophotometric detection was proposed for the determination of nicotinamide (NAM) in pharmaceutical formulations. In this system cyanogen chloride formed from the combination of an acidic KSCN with the NaClO streams reacts with injected NAM to form glutaconic aldehyde. Then the product of these three components was coupled with another buffered (pH 3.5) stream of barbituric acid and directed towards the detector. A 45 s after sample injection the pump was stopped for 130 s. During this time the reactants in the flow cell were provided with the required temperature (40 °C) by placing the cell in a home made cell jacket to increase the yield of the polymethine dye product. Eventually, the absorbance of the formed pink color dye was monitored spectrophotometrically at 560 nm and NAM in the concentration range of 1.0–25.0 μg/mL (R = 0.9974 and D.L = 0.5 μg/mL) was determined. The results obtained by this method were compared statistically and agree with those obtained by the method described in the British Pharmacopoeia.

1. Introduction

Nicotinic acid (NA) and nicotinamide (NAM), also called vitamin B_3, are required for cell respiration, for proper circulation and healthy skin, for the functioning of the nervous system and for the normal secretion of bile and stomach fluids (Capella-Peiró et al., 2004). NAM also occurs in the diet; it is readily deaminated in the body and therefore is nutritionally equivalent to NA (Champe and Harvey, 1994). Moreover, NAM is the active form which functions as a constituent of coenzymes NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide adenine dinucleotide phosphate). All animal species are able to synthesize the metabolically active forms of niacin from an essential amino acid named tryptophan (Valls et al., 2000).

Since approximately 90% of the synthetic vitamin mixtures contain NAM rather than NA, it is highly desirable to have a direct quantitative method for the analysis of vitamin mixtures for NAM. Consequently, many analytical methods have been developed and performed for the quantitative determination of

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such vitamins in different samples, among these methods chromatographic (Capella-Peiró et al., 2004; Valls et al., 2000; Follish et al., 1951; Ismaiel and Yassa, 1973; Kohn et al., 1986), spectrophotometric (Lamb, 1943; Sweeney and Hall, 1951; Holman, 1954) and fluorometric (Zebin et al., 2001; Chaudhuri and Kodicek, 1949; Banerjee et al., 2003) methods were more common. In this work multifarious favorable features are applied to the development of a method that are facile, sensitive, selective and low-price, for the determination of nicotinamide, which present as a main component in all multivitamin formulations. This method can simply apply in any pharmaceutical manufactures and drug quality control laboratory.

2. Experimental

2.1. Apparatus

The constructed stopped-FI system designed in this work (Fig. 1I) consists of a multichannel peristaltic pump (DESAGA Heidelberg-England, with six channels) to propel the flow streams. A six-way injection valve (Rheodyne – USA) was used to inject the NAM samples. Glass bead reactors (10.0 cm length and 2.0 mm i.d.) were used as a reactor or to permit an adequate mixing between different reactants in the streams. A flow cell with 100.0 μL capacity (Starna-micro-flow cell) was placed in a home made cell jacket made of a copper sheet (Fig. 1II), for temperature control. The formed pink color was detected at 560 nm using JENWAY 6405 UV–vis spectrophotometer.

2.2. Reagents and samples

All reagents were of analytical grade and deionize-distilled water (DDW) was used for the preparation of their solutions.

- *Acidic potassium thiocyanate solution*: to prepare 6.0×10^{-2} M stock solution of this reagent, 0.583 g of the KSCN (Fluka) was weighed and dissolved in a small portion of 0.02 M acetic acid and then the volume was completed to 100.0 mL with the same acid solution.
- *Buffer solution*: a buffer mixture of 0.1 M $NaH_2PO_4 \cdot 2H_2O$ (BDH) and 0.4% (w/v) citric acid (BDH) was prepared by dissolving 15.601 g of NaH_2PO_4 and 4.0 g of citric acid in a small fraction of DDW, then transferred to a 1.0 L volumetric flask and diluted to the mark with DDW.
- *Barbituric acid (B-acid)*: 0.25% (w/v) was prepared by dissolving 0.250 g of the barbituric acid (BDH) in sufficient

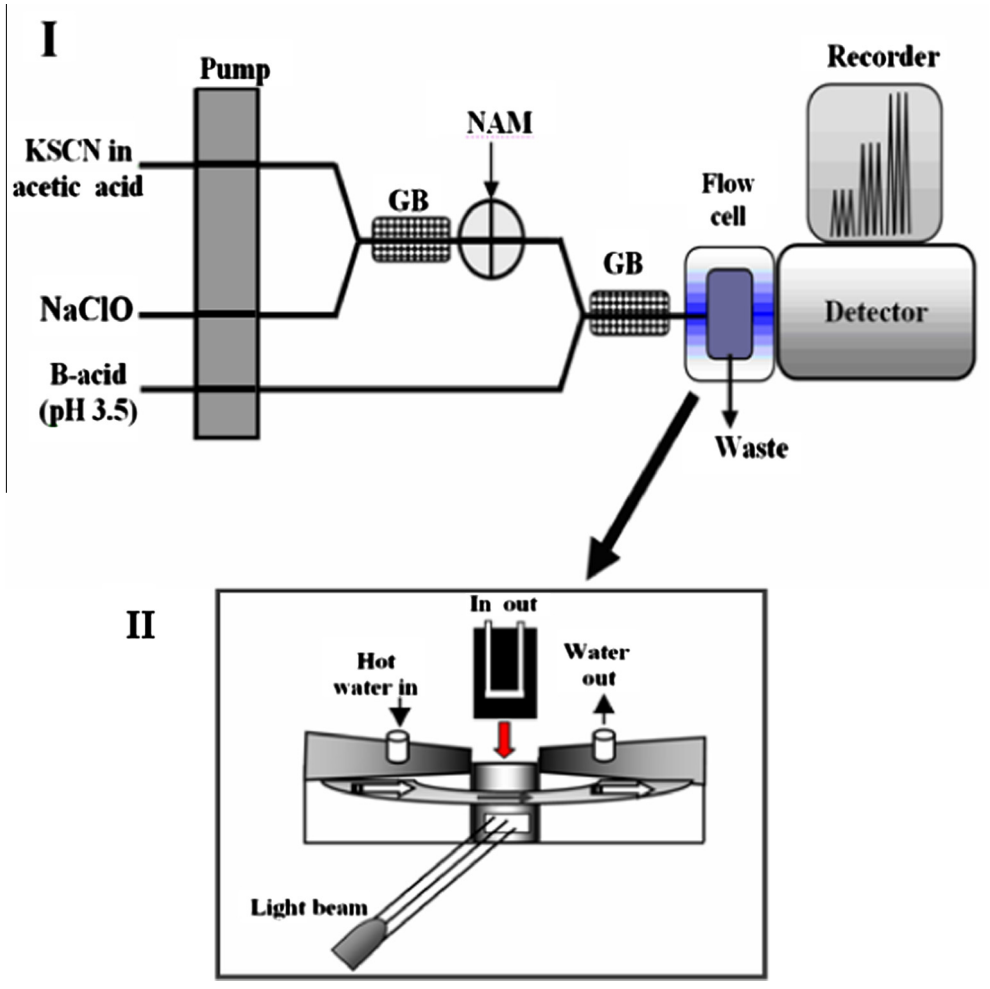

Figure 1 (I) Schematic diagram of the proposed stopped-FI system used for the determination of NAM. (II) Schematic diagram of home made cell jacket unit.

buffer solution, the resulted mixture was quantitatively transferred to a 100.0 mL volumetric flask and diluted with the same buffer solution, then the pH was adjusted to 3.5 with 0.1 M of HCl or NaOH solution.

- *Sodium hypochlorite solution*: a 1.5×10^{-2} M of NaClO solution was prepared by diluting 1.5 mL of NaClO (0.5 M in 0.1 M NaOH, BDH) with DDW to a final volume of 50.0 mL. This solution is prepared daily and standardized iodometrically.
- *Standard nicotinamide solution*: highest purity nicotinamide (BDH) was made up in DDW to a concentration of 100.0 μg/mL, by dissolving 0.1 g of the solid primary standard in a sufficient amount of DDW and diluted to 1.0 L in a volumetric flask with DDW. Desired concentrations were obtained by diluting the stock solution during an investigation.
- *Sample preparation*: solutions of the multivitamin drugs (Table 1) purchased from local drug stores were prepared as follows prior to analysis.

  Tablets: 10 weighed tablets of the multivitamins (one dosage unit) were placed in a mortar then grounded and homogenized to a fine powder. Accurately, a portion equal to the weight of one tablet was weighed, dissolved in a suitable volume of DDW and stirred by using an ultrasonic bath until the material is either dissolved completely in solution or uniformly dispersed then allowed settling. This solution was filtered through a Whatman No. 1 filter paper and transferred into a 100.0 mL volumetric flask.

  Capsules: 10 multivitamin capsules were quantitatively emptied, mixed and weighed; an average weight of one capsule was dissolved in a small portion of DDW and then treated in a similar manner to the tablets.

  Injections: The solution of the injections was prepared for analysis by mixing the content of 10 ampoules and a volume of the resulted mixture equal to that of one ampoule was transferred into a 100.0 mL volumetric flask and diluted to the mark with DDW.

  Syrups: A volume of syrup, equal to that of one dosage (5 mL), was dissolved in a small fraction of DDW and treated in a similar manner to that used for injections.
- *Interfering solutions*: stock solutions for diverse interferences, as they are labeled and expected to be present in the pharmaceutical samples used in this investigation, were prepared as described in Table 2. Other solutions were prepared by serial dilutions of the stock solutions.

3. Procedure

The FIA manifold of the present study was designed depending on the sequences of the slow König reaction shown in Fig. 2.

Accordingly, the flow system consists of three stream lines (Fig. 1); the first one to deliver an acidic potassium thiocyanate solution and when it merges with the hypochlorite solution stream cyanogen chloride is generated. NAM reacts with the formed cyanogen chloride when it is injected into this combined line to form glutaconic aldehyde as a product. The product of these three components was coupled with buffered

Table 2 Preparation of interference stock solutions (1000.0 μg/mL).

Species	Weight amount (mg /100.0 mL)	Supplier
Riboflavine (B_2)	100.0	Schuchardt München
Thiamin (B_1)	100.0	Fluka
Nicotinic acid	100.0	BDH
Pyridoxine (B_6)	100.0	SDI
Glucose	100.0	BDH
Fructose	100.0	Fluka
Saccharine	100.0	Fluka
Sucrose	100.0	Fluka
Dextrose	100.0	Fluka
Benzoic acid	100.0	Fluka
Folic acid	100.0	SDI
Ascorbic Acid	100.0	BDH
Zn^{2+} ($ZnSO_4 \cdot 7H_2O$)	440.0	Fluka
Fe^{2+} ($FeCl_2 \cdot 4H_2O$)	355.0	BDH
Fe^{3+} ($Fe(NO_3)_3 \cdot 9H_2O$)	723.0	Fluka

Table 1 Multivitamin product samples.

Trade name and company	Composition (mg per tablet or capsule or injection or 5 mL of syrup)
Multivitamin orange tablets – Germany (T&D Pharma GmbH)	NAM (18), vitamin C (60), vitamin E (10), vitamin B_5 (6), vitamin B_6 (2) vitamin B_2(1.6), vitamin B_1 (1.4), folic acid (0.2), biotin (0.15), vitamin B_{12} (0.001)
Nuravit tablets – Bulgaria (Eurostock Ltd.)	NAM (5), vitamin A (150 μg), vitamin C (25), vitamin E (5), vitamin B_5 (5), vitamin B_6 (0.6) vitamin B_2 (0.6), vitamin B_1 (0.5), folic acid (0.04), biotin (0.0225), vitamin B_{12} (0.00025), vitamin D (0.00188)
Vitamin B-complex Capsules – India (Brawn laboratory Ltd.)	NAM (10), vitamin B_5 (3), vitamin B_6 (1) vitamin B_2 (2), vitamin B_1 (5), vitamin B_{12} (0.010)
Mephamin vitamin B-complex injection – India (Shaphar)	NAM (50), vitamin B_5 (1), vitamin B_6 (2) vitamin B_2 (2), vitamin B_1 (20)
Bicomed tablets – UK (Medico Remedes PVT. Ltd.)	NAM (15), vitamin B_6 (0.5), vitamin B_2 (1), vitamin B_1 (1)
Levitone syrup – UK (Medico Remedes PVT. Ltd.)	NAM (15), vitamin D_3 (200 IU), vitamin B_6 (1.34), vitamin B_2 (2), vitamin B_1 (2), vitamin B_{12} (0.0025), Dpanathenol (1)
Elvtun tablets – UK (Northwind pharmaceutical compony Ltd.)	NAM (15), vitamin A (2000 IU), vitamin D_3 (200 IU), vitamin B_6 (0.5), vitamin B_2 (1), vitamin B_1 (1)

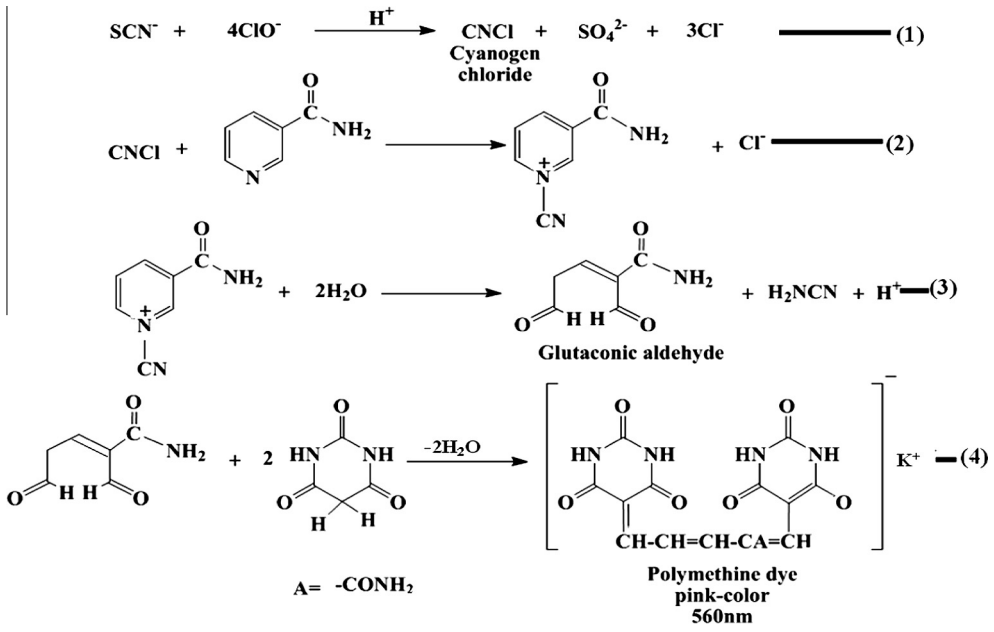

Figure 2 König reaction mechanism with the reactants used in this study.

B-acid stream, then directed towards the flow cell in the detector and the pump was stopped. During this time, the reactants in the flow cell were provided with the required temperature to increase the yield of the colored product and the absorbance was monitored spectrophotometrically at 560 nm.

4. Results and discussion

4.1. Absorption spectra

The absorption spectrum of the colored product obtained according to the preliminary conditions used in manual method for the determination of 14.0 μg/mL NAM is demonstrated in Fig. 3. A pink color dye which is formed at the end of the reaction shows a maximum absorption at 560 nm against the reagent blank.

4.2. Effect of the acid concentration

Preliminary experiments demonstrated that the reaction between thiocyanate and hypochlorite was performed more efficiently in a slightly acidic medium. Moreover, previous investigations have shown that the intensity as well as the stability of color developed is markedly influenced by the pH of the solution (Lundquist et al., 1979; Tanaka et al., 1992).

Therefore, the effect of acetic acid concentration in the range of 0.1×10^{-2}–10×10^{-2} M was studied and the results are illustrated in Fig. 4. Using acetic acid solution with a concentration of 2.0×10^{-2} M gave the maximum peak height. Higher concentrations of acid causes fading of the dye color. Therefore, this concentration was chosen as an optimum.

4.3. Effect of potassium thiocyanate

Different concentrations of potassium thiocyanate solution in the range of 5.0×10^{-3}–1.5×10^{-1} M were tested. As shown in Fig. 5, increases in the absorbance measurements of the dyestuff product were observed by increasing the concentration of thiocyanate. This may be attributed to the instantaneous reaction of SCN^- with ClO^- in a way with increasing its concentration the side reaction of ClO^- with NAM is prevented

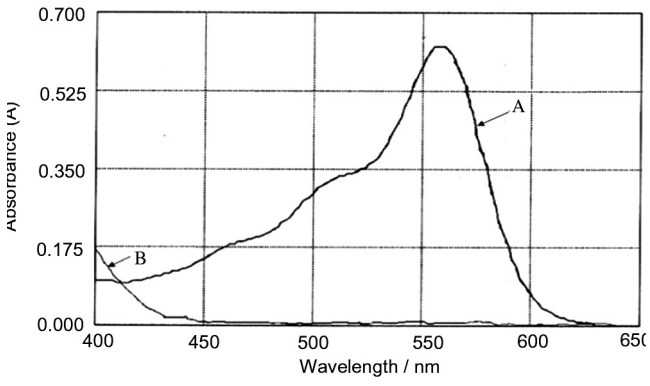

Figure 3 Absorption spectra for the: (A) polymethine dye of 14.0 μg/mL NAM. (B) Blank solution.

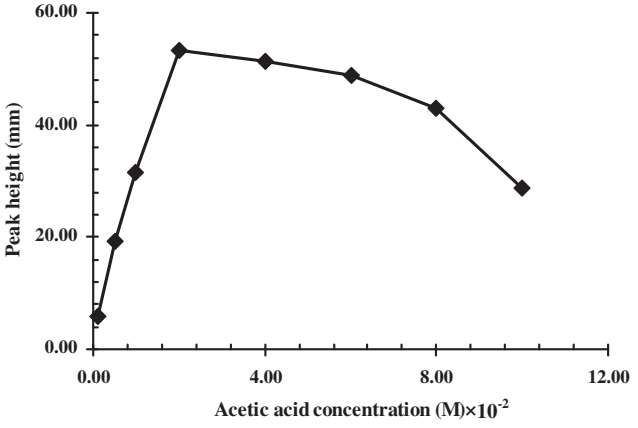

Figure 4 Effect of acetic acid concentration on the peak height.

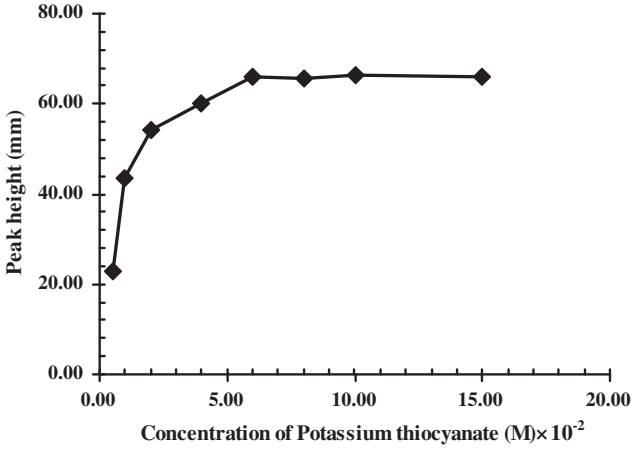

Figure 5 Effect of potassium thiocyanate concentration.

(Holman, 1954), at higher SCN^- concentrations the absorption signal intensity remains constant. Optimum concentration, giving maximum intensity of color, was obtained when thiocyanate salt concentration is equal to 6.0×10^{-2} M. Therefore, this concentration was selected for the subsequent work.

4.4. Effect of sodium hypochlorite

Solutions of sodium hypochlorite in the concentration range from 5.0×10^{-4} to 2.0×10^{-2} M were tested to find the optimum. Fig. 6 shows that the color dyes absorbance in the form of peak height was increased as the concentration of NaClO increased up to 1.5×10^{-2} M. A decrease in the absorbance was observed beyond this concentration. Thus, 1.5×10^{-2} M NaClO solution was selected as an optimum concentration.

4.5. Effect of B-acid reagent

Barbituric acid, among a large number of miscellaneous tested chromogenic reagents, was chosen, since the color formed is relatively stable particularly when it is protected from light and its intensity is unusually high. Moreover, the blank for the reagents (i.e. in the absence of the NAM) is completely colorless and contains non hazardous compounds. The stability and sensitivity of the proposed method using B-acid could be attributed to the fact that the acid can react with glutaconic aldehyde, where tautomeric forms lose methylene hydrogen yielding a structure stabilized by the resonance (Lambert et al., 1975).

Effect of different concentrations of B-acid (0.05–0.35% (w/v)) as a color reagent on the absorbance peak height obtained, with 10.0 μg/mL NAM, was summarized in Fig. 7. This figure shows that maximum absorbance arises when B-acid concentration was 0.2% (w/v), therefore, this concentration was chosen as an optimum concentration.

4.6. Effect of pH

The influence of pH in the range 2.0–5.0 was investigated by measuring the peak height of 10.0 μg/mL NAM, using either 0.1 M HCl or 0.1 M NaOH to adjust the pH of the chromogenic reagent solution. Results shown in Fig. 8 indicate that this system is pH dependent and a great increase in the signal can be observed as the pH raised from 2.0 to 3.5. Maximal absorbance (as peak height) can be obtained when pH is 3.5, while lower and higher pH values show small absorption peak height signals. Therefore, the selected pH in this study was 3.5.

4.7. Temperature effect

Two ways were employed in this study to provide the required temperature. One by using a thermostated 100.0 cm reacting

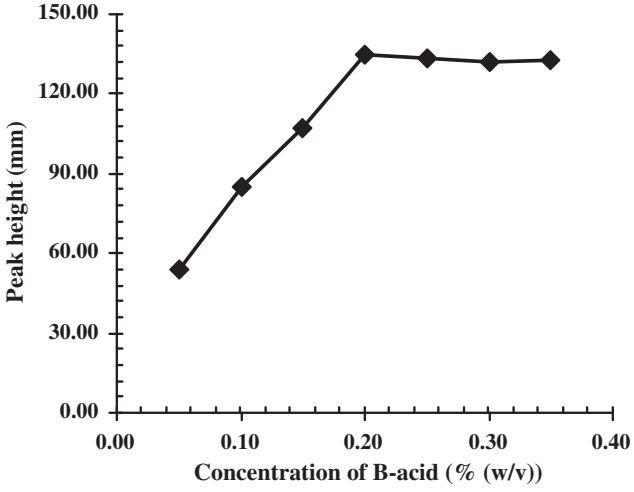

Figure 7 Effect of B-acid concentration.

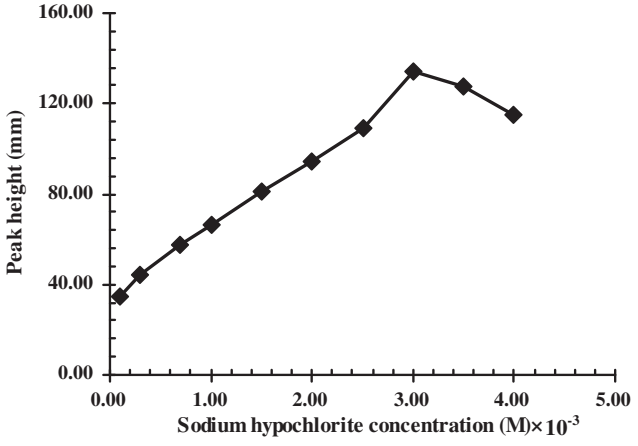

Figure 6 Effect of NaOCl concentration

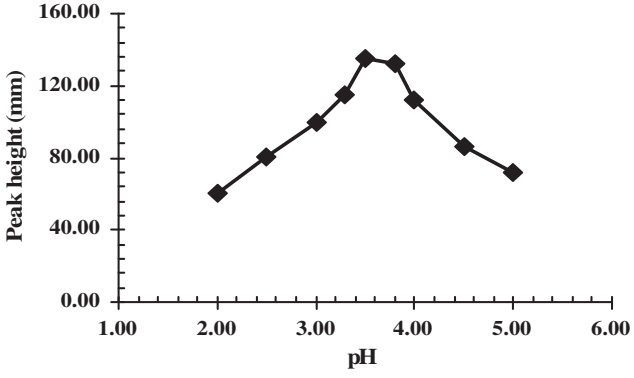

Figure 8 Effect of reagent pH on the represented method.

coil and the pump was stopped as the reactant reached the coil (placed in water bath) for a definite time and re-started again to carry the formed color to the flow cell where it is detected. In the other test, the pump was stopped and the reactants mixture plug reached the flow cell, during the stopping period the flow cell heated to the required temperature using a home made cell jacket (Fig. 1II). In comparing both cases, it was noted that greater drawbacks were observed in applying the first one with respect to the sensitivity, this may be attributed to the large dispersion of the colored product in 100.0 cm coil length and to the formation of air bubbles in the flow system at temperatures greater than 35 °C. Therefore, the second method was applied to study the effect of the temperature on the formation of the colored products. Accordingly, the influences of different temperatures ranged from 25 to 60 °C on the colored product formation have been tested and 40 °C was selected as an optimum reaction temperature depending on the results shown in Fig. 9.

4.8. Effect of system flow rate

Effect of different flow rates (0.5–2.2 mL/min) on maximizing the absorption signal (as peak height) was studied and 1.0 mL/min was selected as an optimum.

4.9. Mixing reactors

In this work two mixing reactor minicolumns packed with glass beads (100–200 mesh) were used. One of them is placed before the injection unit in which the acidic thiocyanate with hypochlorite streams are mixed, while the other is placed before the detection unit. Thus, effects of different lengths (0.0–12.0 cm, 2.0 mm i.d.) of these reactors were examined. The optimum reactor length of the first reactor was found to be 7.0-cm which was sufficient to produce an efficient mixing of both streams, while for the other reactor a column with 10.0-cm length was an optimum.

4.10. Effect of the sample volume

The effect of the sample volume was examined in the range of 40.0–200.0 μL and 120.0 μL was chosen as the most appropriate volume in this study.

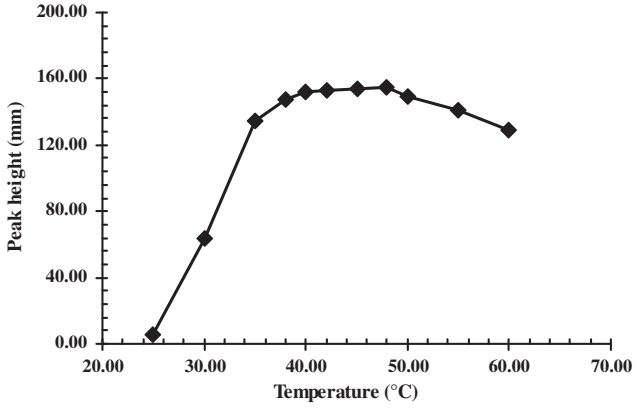

Figure 9 Effect of reaction temperature.

4.11. Optimization of the stopping time period

The time intervals between stopping and restarting the pump flow, in the range 80.0–200.0 s, were optimized, while the pump was stopped as the sample zone reached the flow cell after 45.0 s. From the result shown in Fig. 10, increasing the stopping time gives the reaction sufficient time for completeness which enhances the sensitivity of the developed method. These increments have somewhat little effects after 130.0 s which makes this time sufficient for the subsequent work and selected as an optimum interval time.

Eventually, optimum working conditions employed in this study for the determination of NAM by stopped-FIA are illustrated in Table 3.

5. Calibration graph

To determine the response linearity of the system for the determination of NAM, different concentrations of the analyte were injected. The calibration graph was linear in the range of 1.0–25.0 μg/mL, with a correlation coefficient $R = 0.9974$ and a detection limit equal to 0.5 μg/mL, as shown in Fig.11.

The reliability of the method under optimum experimental conditions was tested by measuring the relative error percent ($E\%$) and the relative standard deviation percent (RSD%)

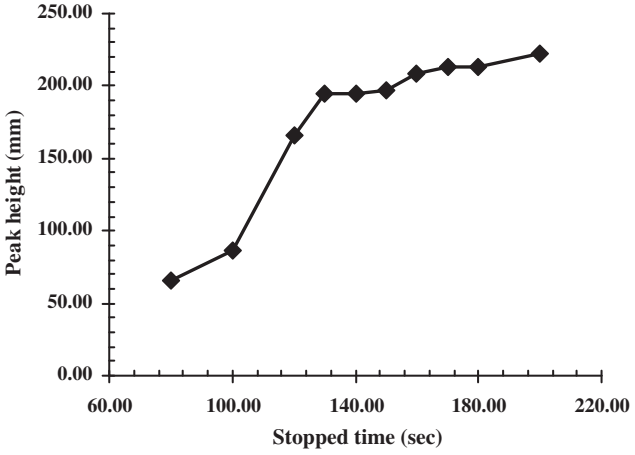

Figure 10 Effect of stopping period time.

Table 3 Optimum experimental conditions used in this investigation.

Parameters	Value
Acetic acid	2.0×10^{-2} M
Sodium hypochlorite	1.5×10^{-2} M
Potassium thiocyanate	6.0×10^{-2} M
B-acid	0.2% (w/v)
pH	3.5
Temperature	40 °C
Flow rate	1.0 mL/min
Length of mixing reactor coils 1 & 2	7.0 and 10.0 cm
Sample volume	120.0 μL
Time interval after injection at which pump is stopped	45.0 s
Stopping time	130.0 s

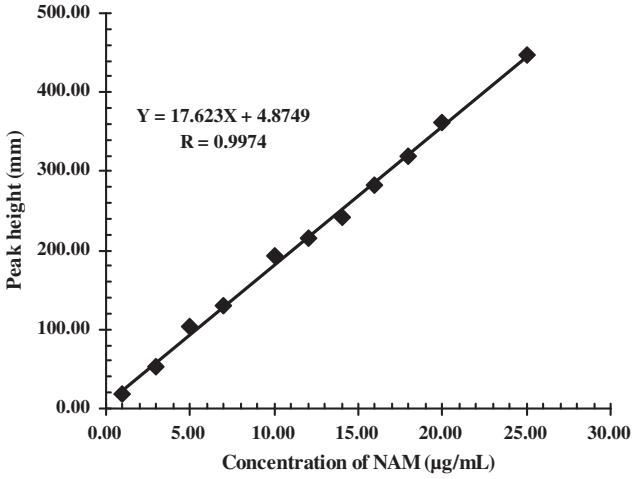

Figure 11 Calibration graph for the determination of NAM.

Table 4 Precision and accuracy measurements of the present method.

Analyte	Analyte concentration (µg/mL)		RSD%	*E*%
	Standard solution	Calculation from proposed method		
NAM	4.00	4.12	1.4	3.00
	8.00	8.22	1.1	2.75
	16.00	15.99	0.3	−0.06

for three different NAM concentrations 4.0, 8.0 and 16.0 µg/mL, located in the calibration range 1.0–25.0 µg/mL and the results are tabulated in Table 4. Satisfactory accuracy and precision of the procedure were confirmed, which demonstrate the performances and legality of the developed method.

6. Interferences study

The interferences of commonly associated excipients, some metal ions and different vitamins were investigated to calculate the capability of the method for the determination of NAM in different common multivitamin products. This study was performed by preparing a synthetic mixture for each of the interfering species mentioned in Table 2 in different excess folds and 5.0 µg/mL of standard NAM. Depending upon the obtained value of the recovery tabulated in Table 5, the present method was not affected by the interferences under study.

Table 5 Effect of interferences on the present method.

Substances	MAC[a] (µg/mL)	Nicotinamide (µg/mL)		Recovery%	TCR[c]
		Added	Found[b]		
Glucose	50.0	5.0	4.96	99.2	10.0
Fructose	50.0	5.0	5.05	101.0	10.0
Saccharine	50.0	5.0	5.02	100.4	10.0
Sucrose	50.0	5.0	4.99	99.8	10.0
Dextrose	50.0	5.0	5.01	100.2	10.0
Benzoic acid	50.0	5.0	4.98	99.6	10.0
Thiamin	50.0	5.0	4.88	97.6	10.0
Pyridoxine	50.0	5.0	4.95	99.0	10.0
Ascorbic acid	25.0	5.0	4.88	97.6	5.0
Riboflavin	25.0	5.0	5.11	102.2	5.0
Folic acid	25.0	5.0	5.09	101.8	5.0
Nicotinic acid	5.0	5.0	5.13	102.6	1.0
Zn^{2+}	25.0	5.0	5.04	100.8	5.0
Fe^{2+}	5.0	5.0	4.99	99.8	1.0
Fe^{3+}	5.0	5.0	5.11	102.2	1.0

[a] Maximum allowable concentrations.
[b] Mean of three replicate analyses.
[c] TCR: tolerable concentration ratio with no interferences (conc. interferent (µg/mL)/conc. NAM (µg/mL)).

7. Determination of NAM in multivitamin products

The developed method was applied for the determination of NAM contents in different pharmaceutical products that are widespread in local drug stores. The measured NAM contents are listed in Table 6. This table also includes the values obtained during analysis of same samples by using the reference procedure described in British Pharmacopoeia (British Pharmacopoeia, 2009). The values presented on Table 5 reveal

Table 6 Application of the developed method for the assay of NAM in some common drugs.

Samples trade name	Nicotinamide[a] (mg per/tablet or capsule or injection or 5.0 mL of syrup)		*E*%
	Standard method[b]	Detectable amount	
Multivitamin orange tablets – Germany (T&D Pharma GmbH)	17.90	18.20	1.68
Nuravit tablets – Bulgaria (Eurostock Ltd.)	4.98	5.03	1.00
Vitamin B-complex Capsules – India (Brawn laboratory Ltd.)	9.97	10.06	0.90
Mephamin vitamin B-complex injection – India (Shaphar)	49.88	49.10	−1.56
Bicomed tablets – UK (Medico Remedes PVT. Ltd.)	14.87	14.59	−1.88
Levitone syrup – UK (Medico Remedes PVT. Ltd.)	14.88	15.30	2.82
Elvtun tablets – UK (Northwind pharmaceutical company Ltd.)	14.89	15.22	2.22

[a] Average of five replication ($n = 5$).
[b] Standard method (HPLC).

a good agreement between the proposed method and the reference one.

8. Conclusion

Pharmaceutical analysis is one of the most important fields in analytical chemistry. The discovery of new drugs and the ongoing update of international regulations for the safety and efficacy of pharmaceutical formulations demand the continuous development of new analytical methods. In addition, new product development, validation of manufacturing process and routine quality control involve the analysis of a considerable amount of samples in order to ensure compliance of the formulations to the limits established by international authorities. Typical quality control tests that involve the analysis of many samples include: (1) Assay of final products. (2) Dissolution profiles during production and validation of manufacturing processes. (3) Dosage uniformity tests. (4) In-process blending uniformity for solid and semi-solid formulations (Sweeney and Hall, 1951). Also, unmonitored storage conditions of the pharmaceutical products further increased the loss of their therapeutic ability. However, drugs quality control has become extremely important from industrial and environmental view points.

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