ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Simultaneous Determination of Naphazoline and Antazoline in Eye Drop Formulations using Net Analyte Signal Standard Addition Method (NASSAM) and Partial Least Squares (PLS).

Reza Hajian[1]*, Vali Zare Shahabadi[2], Abdolmohammad Ghaedi[1], Hadiss Izadi[1] and Mitra Zafari[1].

[1]Gachsaran Branch, Islamic Azad University, 75818-63876, Gachsaran, Iran.
[2] Department of Chemistry, Mahshahr Branch, Islamic Azad University, 63519, Mahshahr, Iran.

ABSTRACT

Two chemometrics methods consisting of net analyte signal standard addition method (NASSAM) and Partial least-squares algorithm (PLS)-1 presented for the simultaneous spectrophotometric determination of Naphazoline (NAP) and Antazoline (ANT) with overlapping spectra. NASSAM combines the advantages of the standard addition method with the net analyte signal concept which enables the extraction of information concerning a certain analyte from spectra of multi-component mixtures. This method has some advantages such as the use of a full spectrum realization, therefore it does not require calibration and prediction steps and only a few measurements are required for the determination. In PLS-1, agreement between predicted and experimental concentrations was fair (r> 0.99 for NAP and ANT models). The RMSE for prediction set were 8.93 and 13.06 for naphazoline and antazoline respectively. Therefore, NASSAM is a better model for simultaneous determination of Naphazoline and Antazoline in the presence of each other in comparison with PLS-1. The simultaneous determination of naphazoline and antazoline was performed in Britton-Robinson buffer (pH 9.0) in the concentration ranges of 1.0×10^{-6} to 2.0×10^{-4} mol L^{-1}.

Keywords: Antazoline, Eye drop, Naphazoline, NASSAM, PLS-1

Corresponding author

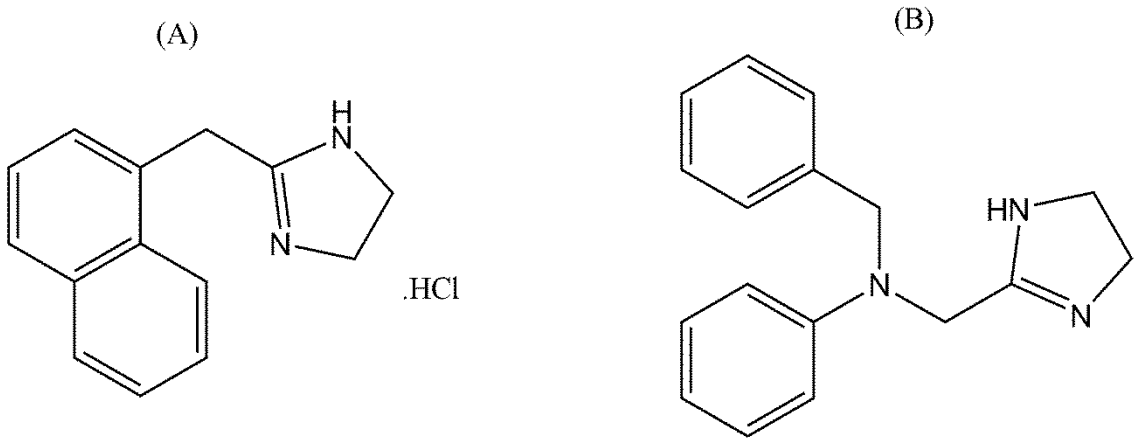
ISSN: 0975-8585

INTRODUCTION

Direct UV-absorbance measurement is subjected to interference from co-formulated drugs, excipients and/or degradation products. Net analyte signal (NAS) is an analytical technique of great utility for extracting quantitative information from spectra composed of unresolved bands. It tends to emphasize subtle spectral features by representing them in a new and visually more accessible way allowing the resolution of multicomponent systems and minimizing the effect of spectral background interferences in pharmaceutical application [1-7].

Naphazoline hydrochloride (NAP) [2-(1-naphthylmethyl)-2-imidazoline monohydrochloride] (Fig. 1A) is a sympathomimetic, which belongs to the imidazole group. It is a vasoconstrictor of relatively long-lasting action that acts on the receptors of the vascular smooth muscle [8]. Antazoline (ANT) is another imidazoline ligand which has alpha 2-adrenoceptor antagonistic properties (Fig. 1B). It is now widely recognized that imidazoline derivatives provided with 2-adrenoceptor antagonist properties not only exhibit a high affinity for 2- adrenoceptors but also bind to non-adrenergic imidazoline sites in various tissues [9]. Many pharmaceuticals containing two active ingredients are currently commercialized in our country as eye drops.

Fig. 1- Chemical structure of (A) Naphazoline hydrochloride and (B) antazoline.



Several methods are available for the determination of naphazoline such as heavy-atom induced room-temperature phosphorescence [10], atomic absorption and emission [11], capillary electrophoresis in the presence of its degradation products [12] and first-derivative UV spectrophotometry [13]. Some chromatographic methods have been also reported for the separation or quantitative measurement of naphazoline, *e.g.,* GC–MS [14], sequential injection chromatography [15] and capillary electrophoresis methods [16]. Literature survey showed few old reports on the simultaneous spectrophotometric analysis of naphazoline and antazoline in drops based on two-component spectrophotometric methods using orthogonal functions [17,18]. Derivative spectrophotometry [19,20], Gas chromatographic and high performance liquid chromatographic methods were also reported for determination of naphazoline and antazoline in pharmaceutical preparations [21-23]. These methods are complicated and need the expensive instruments. The main

disadvantages of derivative methods are their dependence on instrumental parameters like speed of scan and the slit width. The instrumental conditions of recording parent zero-order spectrum have strong influence on the shape and intensity of its derivative generations.

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Coupling of these not-expensive methods with chemometrics methods has circumvented the problems from overlap of spectral data and has made it possible to determine a number of desired analytes in many complex samples, without need for tedious pretreatment or separation of interferences.

In this work, NASSAM and PLS-1 as two powerful chemometrics methods used for simultaneous spectrophotometric determination of naphazoline and antazoline in some binary mixtures and pharmaceutical formulations (eye drop) without requiring any prior separation or derivatization step.

Theory of NASSAM

The net analyte signal (NAS) was defined by Lorber [24], based on spectroscopic methods, as the part of the spectrum of a mixture that is unique for the analyte of interest, i.e., it is orthogonal to the spectra of the interferences.

The conventional notation has been used throughout the following discussion. Boldface capital letter is used for a matrix, a boldface lower case for a column vector and lightface lower case italic for a scalar. The superscript T designates the operation of the vector or matrix transposition and the superscript + denotes the pseudo-inverse of a non-square matrix. The digitized spectrum is referred to as a spectrum vector or simply as a vector, while a spectrum vector of a pure component is called a component vector.

Consider a synthetic mixture containing X and Y with the concentrations of C_X^0 and C_Y^0. The simultaneous determination of two analytes by NASSAM requires having spectrum vector of the binary mixture and the spectra after each standard addition on the mixture. The resulting absorbances are measured and expressed by the following equations:

$$A_0 = \varepsilon_X C_X^0 + \varepsilon_Y C_Y^0 \qquad (1)$$

$$A_1 = A_0 + \varepsilon_X C_{X,s_1} + \varepsilon_Y C_{Y,s_1} \qquad (2)$$

$$A_i = A_{i-1} + \varepsilon_X C_{X,s_i} + \varepsilon_Y C_{Y,s_i} \qquad (3)$$

$$A_n = A_{n-1} + \varepsilon_X C_{X,s_n} + \varepsilon_Y C_{Y,s_n} \qquad (4)$$

where A_0 and A_i are the absorbances of the synthetic mixture before and after of standard additions. The NAS vectors for X and Y after each standard addition, $NAS_{X,i}$ and $NAS_{Y,i}$ can be found by the following equations respectively:

$$NAS_{X_i} = (I - S^{+}S)A_i \quad (5)$$

$$NAS_{Y_i} = (I - T^{+}T)A_i \quad (6)$$

Where I is an identical matrix, S and T are the matrixes of absorbances in different concentrations of interference (X or Y) respectively. By definition, it is always possible to split up the spectrum of a sample (A_i) into two distinct parts for example: NAS_X which is orthogonal to the spectra of the interference (Y) and $S^{+}SA_i$. Where $S^{+}SA_i$ is a part of the spectrum that is generated by a linear combination of the spectra of the interfering agent. Consequently, $S^{+}SA_i$ can not be unique for the analyte of interest, because it can also be produced by a mixture of interfering agent. The other part NAS_Y is orthogonal to the spectra of the interference reflecting the part of the spectrum which is only depending on the analyte X present in the mixture.

In binary mixtures when the interferences are known, the NAS can be calculated for the analytes. Norm of the NAS vector can be used to construct a univariate calibration model.

EXPERIMENTAL

Reagents

Naphazoline hydrochloride and antazoline phosphate were kindly provided by Sina Daroo Pharmaceutical Co. (Tehran, Iran). Analytical grade of phosphoric acid, boric acid, acetic acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Britton–Robinson (B-R) buffers (0.1mol L^{-1} in phosphate, acetate and borate) in the pH range of 2-10 were used throughout.

A 1.0 × 10^{-3} mol L^{-1} naphazoline hydrochloride solution was prepared daily by dissolving 0.0247 g of NAP (99.5%) in distilled water and diluted in a 100 ml volumetric flask. These solutions were reserved in a refrigerator at 4^{o}C in dark. A 1.0 × 10^{-3} mol L^{-1} antazoline phosphate solution was prepared daily by dissolving 0.0402 g of ANT (99.5%) in distilled water and diluted in a 100 ml volumetric flask.

Instrumentation and software

UV-Visible absorption spectra were measured on an Agilent UV-Vis spectrophotometer, PerkinElmer (Lambda 25), with the use of 1.0 cm quartz cells.

A Pentium IV (2.53 GHz) computer controlled all the setting and data processing. All spectra were saved in text files and transformed to an in house Matlab program version 7.6.0.324 (R2011a) to calculate NAS vectors.

A pH-meter (Metrohm, Model 827) with a double junction glass electrode was used to determine pH of the solutions.

ISSN: 0975-8585

Preparation of Real Samples

To assay eye drop containing naphazoline hydrochloride (0.05 g) and antazoline phosphate (0.5 g) in each 100 ml of drop, ca.1.0 ml of syrup was transferred into a 100 ml measuring flask and diluted to the mark by doubly distilled water. The absorbances of the solutions recorded after simultaneous standard additions of two analytes after 10 times more dilution.

General Procedure

NASSAM

An aliquot of a solution containing NAP and/or ANT and 1.0 ml Britton–Robinson buffer solution (pH 9.0) added into a 6×25 ml volumetric flasks and made up to the mark with deionized water after simultaneous standard additions of NAP and ANT at the mole ratio of 1:1. The spectrum of each solution recorded in the wavelength range of 240-320 nm and saved as text files. For calculating the norms of NAS vectors for each analyte, the matrixes of S and T as the interference matrices designed in the concentration ranges of 0.5-100.0 mmol L^{-1} (table 1) at the wavelength range of 240-320 nm. The text files for each spectrum transferred to Matlab program for calculating the norms of NAP and ANTA simultaneously (equs. 5, 6). In the end, the concentration of each component calculated by standard addition plot (Norm of NAS vs. added concentrations).

Table 1. Interferent concentrations of naphazoline or antazoline used in matrices “S” and “T”.

Interferent/μmol L^{-1}			
0.5	5.0	35.0	65.0
1.0	10.0	40.0	75.0
1.5	15.0	45.0	80.0
2.0	20.0	50.0	85.0
3.0	25.0	55.0	90.0
4.0	30.0	60.0	100.0

Methodology for PLS regression

Two sets of the standard solutions for two drugs were prepared (24 calibration solutions and 40 validation solutions). The calibration and validation sets were prepared according to 6-level full factorial design. Using such a design, maximum information for each compound can be obtained by using only a few numbers of standard solutions. In Table 2, the concentrations of standard solutions are represented. In order to evaluate the performance of the employed models, 40 standard solutions were considered in the validation set whose concentrations were selected randomly (Table 3). For preparation of each standard solution, appropriate volumes of the stock solution of each drug were added to 10.0 ml volumetric flasks and diluted with distilled water. The absorbance spectra of each solution was recorded in the wavelength region of 240–320 nm and digitized in 1.0 nm intervals. The order of recording of absorbance spectra of calibration and prediction samples were chosen randomly in order to neglect the effect of recording time. The performance of each model was evaluated by root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and the correlation coefficients between the expected values and calculated values for calibration and prediction sets.

ISSN: 0975-8585

Table 2. The binary concentrations of NAP and ANT used for calibration set in PLS-1.

No. of Sample	ANT/μmol L^{-1}	NAP/ μmol L^{-1}
1	0.5	0.5
2	50.0	50.0
3	50.0	5.0
4	0.5	40.0
5	20.0	15.0
6	40.0	30.0
7	30.0	0.5
8	20.0	50.0
9	0.5	15.0
10	20.0	30.0
11	40.0	15.0
12	50.0	30.0
13	15.0	5.0
14	30.0	10.0
15	40.0	40.0
16	50.0	30.0
17	30.0	40.0
18	40.0	5.0
19	15.0	40.0
20	50.0	20.0
21	5.0	50.0
22	30.0	20.0
23	50.0	40.0
24	10.0	5.0

Table 3. The binary concentrations of NAP and ANT used for prediction set in PLS-1.

No. of Sample	ANT/μmol L^{-1}	NAP/μmol L^{-1}	No. of Sample	ANT/μmol L^{-1}	NAP/μmol L^{-1}
1	5.0	0.5	21	30.0	15.0
2	15.0	0.5	22	50.0	15.0
3	20.0	0.5	23	0.5	20.0
4	40.0	0.5	24	5.0	20.0
5	50.0	0.5	25	10.0	20.0
6	0.5	5.0	26	15.0	20.0
7	5.0	5.0	27	20.0	20.0
8	10.0	5.0	28	40.0	20.0
9	20.0	5.0	29	0.5	30.0
10	30.0	5.0	30	10.0	30.0
11	0.5	10.0	31	15.0	30.0
12	50.0	10.0	32	30.0	30.0
13	10.0	10.0	33	5.0	40.0
14	15.0	10.0	34	10.0	40.0
15	20.0	10.0	35	20.0	40.0
16	40.0	10.0	36	0.5	50.0
17	50.0	10.0	37	10.0	50.0
18	5.0	15.0	38	15.0	50.0
19	10.0	15.0	39	30.0	50.0
20	15.0	15.0	40	40.0	50.0

ISSN: 0975-8585

RESULTS AND DISCUSSION

The absorption spectra of NAP and ANT have shown in Fig. 2. As can be seen, the maximum wavelengths of two compounds are close to each other and their spectra have overlap. Therefore, determination of NAP and ANT in the presence of each other is impossible by direct spectrophotometry (beer's law). Therefore, we used NASSAM and PLS as two chemometrics methods for simultaneous determination of NAP and ANT in binary mixtures and pharmaceutical formulations (eye drop).

Fig. 2- Absorbance spectra of naphazoline and antazoline in phosphate buffer pH 9.0.

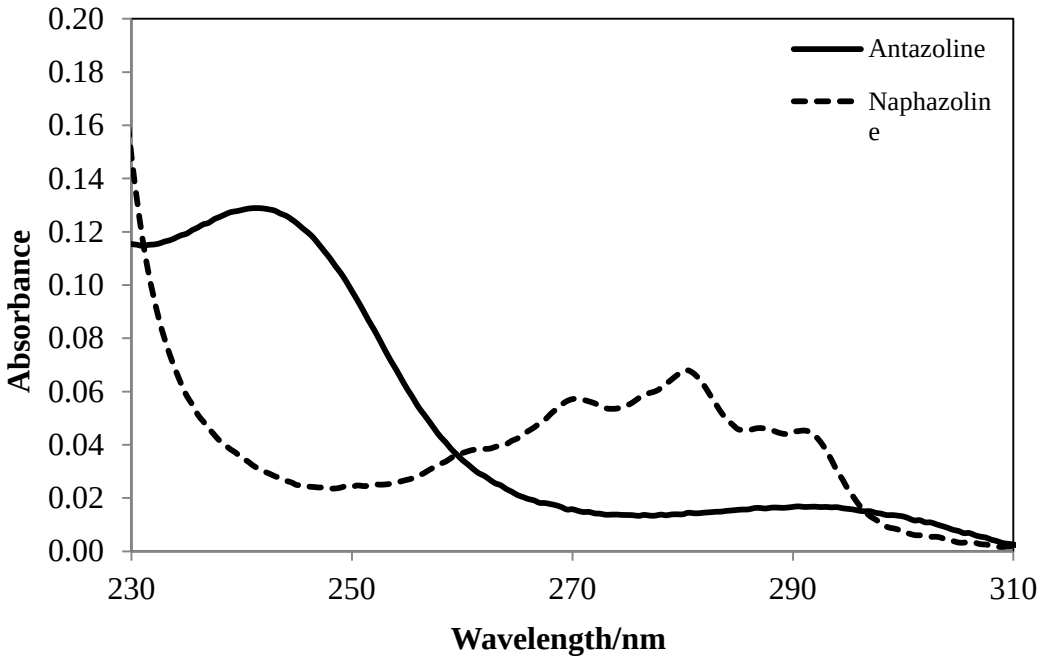


Table 4. Determination of NAP and ANT in some binary mixtures by NASSAM.

	Added/µmol L^{-1}		Found/µmol L^{-1}		Recovery (%)	
Sample	NAP	ANT	NAP	ANT	NAP	ANT
1	5.0	5.0	5.76	5.55	115.20	111.00
2	10.0	10.0	10.00	9.11	100.00	91.10
3	20.0	10.0	20.50	8.01	102.50	80.10
4	30.0	10.0	29.74	7.77	99.13	77.70
5	50.0	10.0	46.92	10.35	93.84	103.50
6	15.0	15.0	14.64	14.75	97.60	98.33
7	20.0	20.0	20.00	20.25	100.00	101.25
8	10.0	30.0	10.87	31.57	108.70	105.23
9	30.0	30.0	33.33	30.89	111.10	102.97
10	10.0	50.0	10.59	43.22	105.90	86.44

ISSN: 0975-8585

Simultaneous determination of naphazoline and antazoline

Analysis in some synthetic and real samples by NASSAM

In order to check the performance of the proposed model (NASSAM), the method was applied for the simultaneous determination of NAP and ANT in ten binary mixtures (table 4) and a commercial formulation (eye drop, table 5). The values of RMSE for naphazoline and antazoline were determined as 0.52 and 0.90 respectively in synthetic samples showing the high performance of the proposed method for simultaneous determination of two components in the binary mixtures. As it has shown in table 5, the results have validated with spiked standard solutions with superior consistency.

Table 5. Determination of NAP and ANT in eye drop, expressed as $\overline{X} \pm (t.s)/\sqrt{N}$, for N=3 measurements and $t_{95\%, (N-1=2)}$ =2.92

Sample	Added/ µmol L^{-1}		Found/ µmol L^{-1}		Recovery (%)	
	NAP	ANT	NAP	ANT	NAP	ANT
Eye drop*	- - - -	- - - -	10.20±0.64	67.24±2.42	----	----
	10.0	10.0	21.19±2.35	78.44±8.71	111.20	109.90
	20.0	20.0	30.21±9.76	85.29±12.51	90.25	100.00

*Eye drop manufactured by Sina Daroo containing NAP (0.05 g) and ANT (0.5 g) in each 100 ml of the solution.

Analysis in some synthetic and real samples by PLS-1

In order to establish the ranges in which linearity between absorbance and concentration exists for the two compounds, different concentration of NAP and ANT were prepared. For each medicine, the maximum absorbance wavelengths were used. The NAP and ANT showed a linear range between 1.0 and 200.0 µmol L^{-1}. The spectral region between 240 and 320 nm was chosen with the wavelength increment of 0.5 nm, because it provides the maximum spectral information of the components in the mixture. A very important parameter in multivariate calibration (using PLS-1 algorithm) in order to achieve the best predictions is the selection of the number of principal components. Thus, the data set was mean centered to build the calibration models and their performances were evaluated by leaving one out cross validation, in which each sample was left out once, and its concentration was estimated by a model built with the remaining samples [25]. In order to find the optimum number of factors for the PLS-1 model the prediction residual error sum of square (PRESS) [25,26] was calculated according to Eq. (1):

$$\mathrm{PRESS} = \sum_{i=1}^{m} (C_i - C_j)^2$$

ISSN: 0975-8585

where m is the total number of samples, C_i is the estimated concentration, and C_j is the reference concentration. It was verified that the optimum number of latent variables using the PLS-1 algorithm was 3 for both models (NAP and ANT), as it yielded the minimum PRESS values.

In order to evaluate the prediction performance of the PLS-1, the constructed models were used for NAP and ANT determinations, so that all the constituents of the samples are known, including the different concentrations of NAP and ANT. Twenty-Five samples containing the same excipients as the mixtures for the calibration set were used. From the experimental and predicted values for prediction set, it can be seen that the RMSE for prediction set were 8.93 and 13.06 for naphazoline (Fig. 3) and antazoline (Fig. 4) respectively.

Fig. 3- The linear correlation between experimental concentrations of naphazoline and estimated values for prediction set calculated by PLS-1.

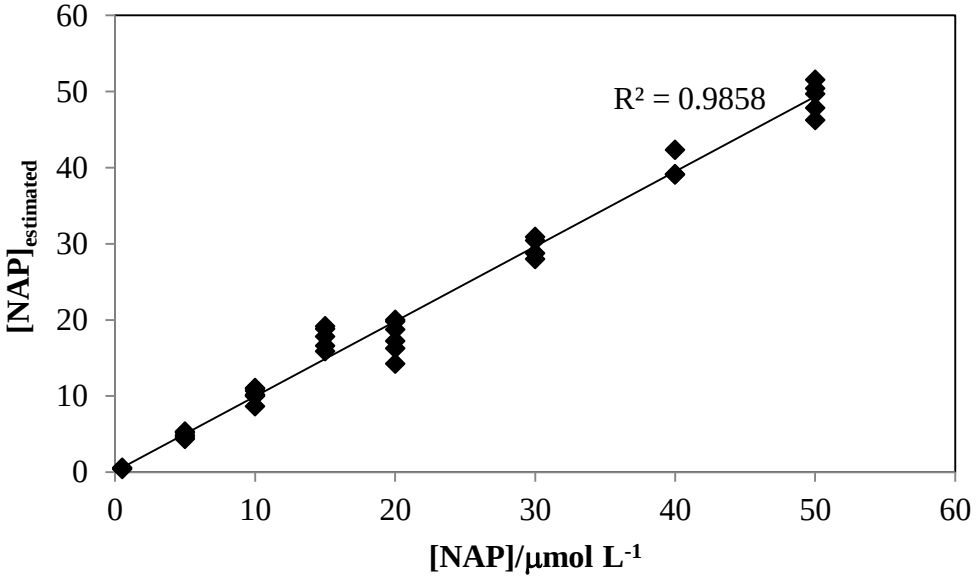
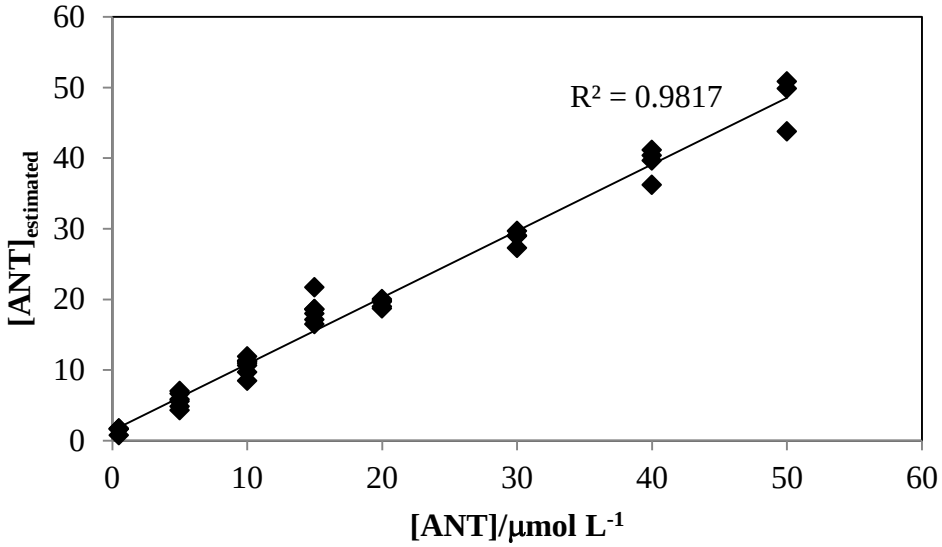


Fig. 4- The linear correlation between experimental concentrations of antazoline and estimated values for prediction set calculated by PLS-1.



ISSN: 0975-8585

CONCLUSIONS

Quantification of naphazoline and antazoline was accomplished from spectrophotometric data by two chemometrics methods based on NASSAM and PLS-1. When the interferents are known, the part of the overlapping spectra orthogonal to the space of interferents can be calculated as the net analyte signal and this is attributed to the analyte concentration. Therefore the analyte concentrations can be determined simultaneously from a unique standard addition plot. Two popular multivariate calibration methods, *i.e*., PCR and PLS, require that the optimum number of factors or principal components are selected. This selection may lead to overfitting and/or underfitting. This study shows that NASSAM is simple, inexpensive, precise and affordable and doesn't require factor selection. Hence, it can be a powerful and substituted method in comparison with PLS-1 for simultaneous analysis of naphazoline and antazoline.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of this work by Gachsaran branch, Islamic Azad University and the assistance of Sina Daroo Pharmaceutical Company for supplying pure drug compounds.

REFERENCES

[1] Galera MM, Zamora DP, Vidal JLM, Frenich AG, Mansilla AE, Munoz de la Pena A, Lopez FS. Talanta 2003; 59: 1107-1116.
[2] Kompany Zareh, M, Mirzaei S. Anal Chim Acta 2004; 526: 83-94.
[3] Hemmateenejad B, Ghavami R, Miri R, Shamsipur M. Talanta 2006; 68: 1222-1229.
[4] Espinosa-Mansilla A, Duran Meras IM, Gomez JR, Munoz de la Pena A, Salinas F. Talanta 2002; 58: 255-263.
[5] Mirmohseni A, Abdollahi H, Rostamizadeh K. Anal Chim Acta 2007; 585: 179-184.
[6] Munoz de la Pena A, Espinosa-Mansilla A, Acedo Valenzuela MI, Goicoechea HC, Olivieri AC. Anal Chim Acta 2002: 463: 75-88.
[7] Asadpour-Zeynali K., Bastami M. Spectrochim Acta Part A 2010; 75: 589-597.
[8] Hillman AG, Rall T, Nier A, Taylor P. The Pharmacological Basis of Therapeutics, 1996, McGraw-Hill, New York.
[9] Berdeu D, Puech R, Ribes G, Mariani MM, Bertrand G. Eur J Pharmacol 1997; 324: 233-239.
[10] Gutierrez AF, Carretero AS, Dıaz BC, Blanco CC. Appl Spec 1999; 53: 741-744.
[11] Khalil S. Mikrochim Acta 1999; 130: 181-184.
[12] Yesilada B, Tozkoparan N, Gokhan L, Oner L, Ertan M. J. Liq Chromatogr & Rel Technol 1998; 21: 2575-2588.
[13] Charles JJ, Bertucat M. Anal Lett 1999; 32: 373-382.
[14] Rossi SS, Molaioni F, Botre FJ. Anal Toxicol 2005; 29: 217-222.
[15] Chocholous P, Satinsky D, Solich P. Talanta 2006; 70: 408-413.
[16] Marchesini AF, Williner MR, Mantovani VE, Robles JC, Goicoechea HC. J Pharm Biomed Anal 2003; 31: 39-46.
[17] Abdine H, Wahbi AM. Korany MA. J Pharm Pharmacol 1972; 24: 522-524.
[18] Wahbi AM. Abdine H, Korany MA, El-Yazbi FA. J Pharm Sci 1978; 67: 140-141.

[19] Souri E, Amanlou M, Farsam H, Afshari A. Chem Pharm Bull 2006; 54: 119-122.
[20] Hajian R, Shams N. Kaedi I. E-J Chem 2010; 7: 1530-1538.
[21] Peat J, Garg U. Methods Mol Biol 2010; 603: 501-508.
[22] Koziol TR, Jacob JT, Achari RG. J Pharm Sci 1979; 68: 1135-1138.
[23] Ruckmick SC, Masch DF, Duong ST. J Pharm Sci 1995; 84: 502-507.
[24] Lorber A. Anal Chem 1986; 58: 1167-1172.
[25] Haaland DM, Thomas EV. Anal. Chem 1988; 60: 1193-1202.
[26] Haaland DM, Thomas EV. Anal Chem 1988; 60: 1202-1208.