ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Simultaneous Determination of Paracetamol, Dextromethorphan and Phenylephrine in Bovine Milk Using Net Analyte Signal Concept in Univariate Calibration,

Reza Hajian[*1] and Somayeh Naraki[2]

[1]Young Researchers and Elite Club, Gachsaran branch, Islamic Azad University, P.O. Box 75818-63876, Gachsaran, Iran
[2]Department of Chemistry, Gachsaran branch, Islamic Azad University, P.O. Box 75818-63876, Gachsaran, Iran

ABSTRACT

A kind of univariate calibration, net analyte signal combined with standard addition (NASSAM) is presented for determination of Paracetamol, Dextromethorphan and Phenylephrine in the presence of each other by spectrophotometry. The method has both advantages of standard addition method and net analyte signal enables the extraction of information concerning an analyte from spectra of multi-component mixtures. The use of full spectrum to calculate net analyte signal vector and also no requirement of calibration and prediction steps are the most important advantages of NASSAM. The simultaneous determination of Paracetamol, Dextromethorphan and Phenylephrine was performed in Britton-Robinson buffer (pH 10). Three pharmaceutical compounds were determined in concentration range of 0.5-190 µmol L^{-1} for Paracetamol and 0.5-900 µmol L^{-1} for Dextromethorphan and Phenylephrine. In this work, the method of NASSAM has been used for determination of Paracetamol, Dextromethorphan and Phenylephrine in some ternary mixtures and bovine milk with satisfactory results.

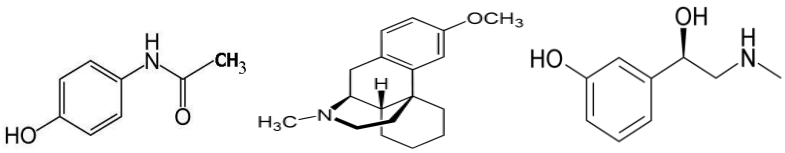
Keywords: Paracetamol, Dextromethorphan, Phenylephrine, NASSAM, Bovine Milk

Corresponding author
E-mail: shishehbor47@iauyazd.ac.ir

ISSN: 0975-8585

INTRODUCTION

Cough and cold pharmaceutical preparations are one of the most extended formulations in the world and have got many pharmaceutical forms; syrup, suspension, sachets, capsules and tablets [1]. These preparations represent complex formulations containing several active ingredients and a broad spectrum of excipients such as flavoring agents, saccharine or aspartame, acidulates, natural or artificial coloring and flavoring agents, dyes, sweeteners and preservatives [2,3]. The majority of these ingredients are present as a mixture of basic nitrogenous amino compounds and their separation in pharmaceutical forms is quite complicated due to similarities of their physical and chemical properties [4]. Paracetamol (PAR) (Fig. 1 A) uses for mild to moderate pain such as headache, toothache, minor pain of osteoarthritis and pain from minor surgery applications [5-7]. Dextromethorphan (DEX) (Fig. 1 B) uses for nonproductive cough caused by colds and flu and is available in the form of syrup, coated tablets and oral drops. This medication through a direct effect on the cough center in the medulla oblongata undermines reflection [7]. Phenylephrine (PHE) (Fig. 1 C), is owned inhibitors cough, sputum, hypnotics, and nasal mucosa.



A B C

Figure 1: Chemical structures of AC (A), DEX (B) and PHE (C).

Several gas chromatographic and high-performance liquid chromatographic (HPLC) [8-10], methods have been described for determination of Dextromethorphan and Paracetamol. Sample preparation is performed by liquid-liquid extraction [8] solid-phase extraction [9] or column switching [10].

In this work, a sensitive, selective, accurate and inexpensive procedure was applied for determination of Paracetamol, Dextromethorphan and Phenylephrine by NASSAM with simultaneous addition of three analytes. This method is a novel method based on the net analyte signal concept for analysis of three active components PAR, DEX and PHE in bovine milk samples. In this paper, an attempt was made to calculate NAS vectors and attribute them to the analyte concentration using UV-visible spectrophotometry technique.

Theory

The net analyte signal (NAS) was defined by Lorber [11,12] based on spectroscopic methods, as the part of a spectrum for a mixture that is independent to all of the interferent spectra, i.e., it is an orthogonal vector to the space of interferences. This method uses the full spectrum data in opposite of H-point standard addition method [13] that only one pair of variables chosen for analysis.

ISSN: 0975-8585

For more understanding about the concept of NAS, consider a synthetic mixture containing PAR, DEX and PHE in concentration of 10 μmol L^{-1} for each analyte. The simultaneous determination of three analytes by NASSAM requires having the spectrum vector of mixture. The standard concentrations of PAR, DEX and PHE are simultaneously added to the sample solution. The spectrum of the mixture after each standard addition is recorded base on the following equations:

$$A_0 = \varepsilon_{PAR} C^0_{PAR} + \varepsilon_{DEX} C^0_{DEX} + \varepsilon_{PHE} C^0_{PHE} \quad (1)$$

$$A_1 = A_0 + \varepsilon_{PAR} C_{PAR,s_1} + \varepsilon_{DEX} C_{DEX,s_1} + \varepsilon_{PHE} C_{PHE,s_1} \quad (2)$$

$$A_i = A_{i-1} + \varepsilon_{PAR} C_{PAR,s_i} + \varepsilon_{DEX} C_{DEX,s_i} + \varepsilon_{PHE} C_{PHE,s_i} \quad (3)$$

$$A_n = A_{n-1} + \varepsilon_{PAR} C_{PAR,s_n} + \varepsilon_{DEX} C_{DEX,s_n} + \varepsilon_{PHE} C_{PHE,s_n} \quad (4)$$

Where A_0 and A_i are the absorbances of the synthetic mixture before and after of standard additions. C^0_{PAR}, C^0_{DEX}, C^0_{PHE} and C_{AC,s_i}, C_{DEX,s_i}, C_{PHE,s_i} are the initial added concentrations of PAR, DEX and PHE to the sample in the i^{th} step. Also ε_{PAR}, ε_{DEX} and ε_{PHE} are molar absorbtivities of PAR, DEX and PHE respectively.

The NAS vectors for PAR, DEX and PHE compounds after each standard addition, $NAS_{PAR,i}$, $NAS_{DEX,i}$ and $NAS_{PHE,i}$ can be found by the following equations respectively:

$$NAS_{PAR_i} = (I - R^+ R)A_i \quad (5)$$

$$NAS_{DEX_i} = (I - S^+ S)A_i \quad (6)$$

$$NAS_{PHE_i} = (I - T^+ T)A_i \quad (7)$$

Where *I* is an identical matrix, *R*, *S* and T are the matrixes of absorbances in different concentrations of both interferences according to table 1. Based on the equations 5 to 7, the spectrum of the mixture (A_i) is the combination of two independent parts i.e., NAS_{PAR}, which is orthogonal to the space of interferences (DEX and PHE) and also (R^+R) A_i. Where (R^+R) A_i is a part of the spectrum that is generated by a linear combination of the spectra of the interfering agents. The superscript "+" indicates the pseudo inverse of a non-square matrix. Consequently, (R^+R) A_i is not independent to the concentration of interferents. The other part NAS_{PAR} is orthogonal to the space of interferences (DEX and PHE). Therefore it is dependent only to the concentration of PAR in the mixture. Therefore the orthogonal vectors of PAR, DEX and PHE which known as NAS_{PAR}, NAS_{DEX} and NAS_{PHE} correlate with the concentrations of analytes (PAR, DEX and PHE) respectively. Fig.2 shows the geometrical presentation of NAS vectors. As it has shown, the norm of the NAS_{PAR} vector depends only to the concentration of PAR in the mixture. Therefore it can be used as a net signal to correlate with analyte concentrations.

ISSN: 0975-8585

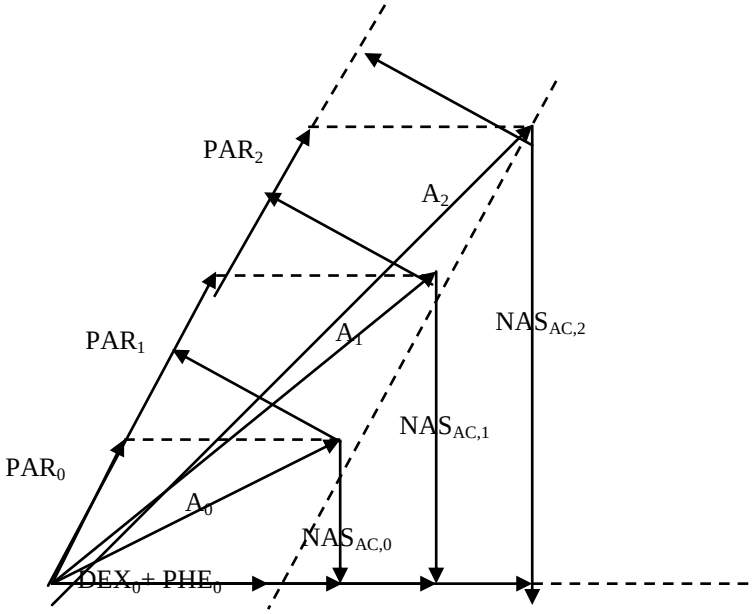


Figure 2: Representation of vector space for analyte (AC) and other analytes (DEX and PHE) in two dimensional. NAS vector NAS_{AC} is different from AC vector in direction and length.

Table 1: The concentrations of PAR, DEX and PHE used in the interferent matrixes.

Concentration (µmol L^{-1})		
PAR	DEX	PHE
0.5	0.5	0.5
1.0	1.0	1.0
5.0	5.0	5.0
15.0	15.0	15.0
30.0	30.0	30.0
50.0	50.0	50.0
70.0	70.0	70.0
100.0	100.0	100.0
130.0	130.0	130.0
150.0	150.0	150.0

In this work, the norm of NAS for each analyte in the ternary mixture is calculated and correlate to each analyte concentration. This correlation is linear and in the case of matrix effect, standard addition plots can be constructed.

EXPERIMENTAL

Initial investigation

To demonstrate the analytical applicability of the proposed method for the analysis of ternary mixtures, three pure spectra of PAR, DEX and PHE were recorded separately. As it has shown in Fig. 3, the spectra are too overlapped in the range of 210–320 nm. Fig. 4 shows successive standard addition of three analytes (PAR, DEX and PHE) at the same mole ratio on the mixture and NAS vectors for components were calculated simultaneously based on Eqs. (5-7). Fig. 5 shows the standard addition plot based on the norms of NAS vectors for

ISSN: 0975-8585

each analyte (PAR, DEX and PHE) that can be used to calculate the simultaneous concentrations of components by interpolation.

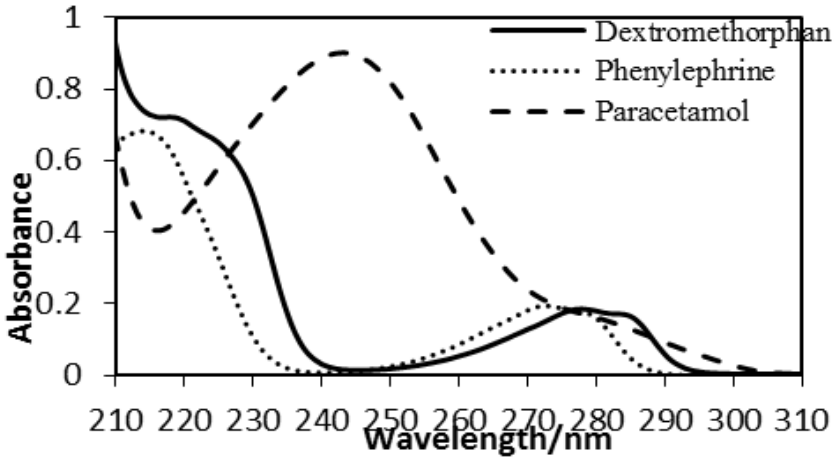


Figure 3: Absorption spectra of 10.0 µmol L^{-1} Paracetamol (a), 10.0 µmol L^{-1} Dextromethorphan (b) and 10.0 µmol L^{-1} Phenylephrine (c) at pH 10.0.

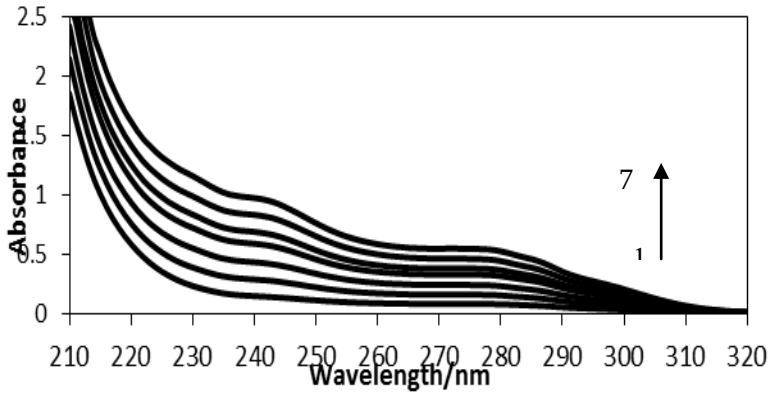


Figure 4: The spectra after addition of standards AC, DEX and PHE in the same mole ratios in the concentration range of 10-60 µmol L^{-1} for spectra 1-7. The initial solution contains a mixture of Paracetamol (10.0 µmol L^{-1}), Dextromethorphan (10.0 µmol L^{-1}) and Phenylephrine (10.0 µmol L^{-1}).

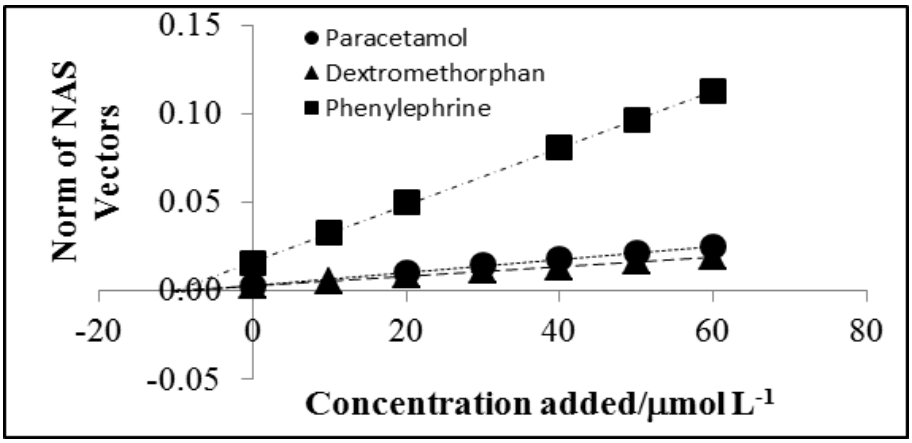


Figure 5: Norms of NASs for components AC, DEX and PHE *vs.* added concentration. All the conditions are as figure 4.

ISSN: 0975-8585

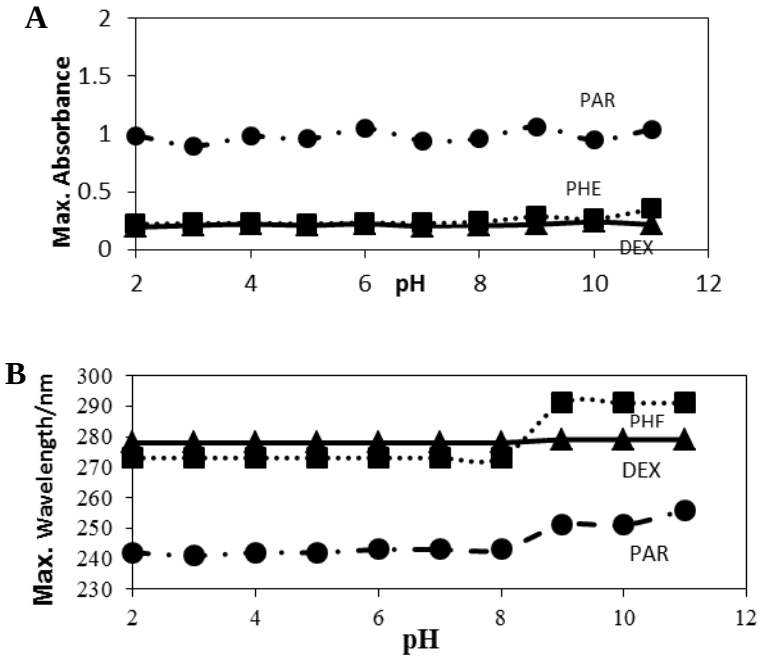


Figure 6: Effect of pH on the maximum absorbance (A) and λ_{max} (B) for AC, DEX and PHE using B-R buffer. Conditions: 10.0 µmol L^{-1} Paracetamol, 10.0 µmol L^{-1} Dextromethorphan and 10.0 µmol L^{-1} Phenylephrine.

Reagents

Paracetamol, Dextromethorphan and Phenylephrine were kindly provided by Bakhtar Bioshimi Co. (Kermanshah, Iran). Analytical grade phosphoric acid, boric acid, acetic acid and sodium hydroxide supplied from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

A 1.0×10^{-3} mol L^{-1} Paracetamol solution was prepared daily by dissolving 0.0075 g paracetamol (99.0%) in distilled water and diluted in a 50 ml volumetric flask to the mark. A 1.0×10^{-3} mol L^{-1} dextromethorphan hydrochloride solution was prepared daily by dissolving 0.0185 g of DEX (99.5%) in water and diluted in a 50 ml volumetric flask. A 1.0×10^{-3} mol L^{-1} Phenylephrine solution was prepared daily by dissolving 0.0120 g of PHE (99.5%) in distilled water and diluted into a 50 ml volumetric flask. These solutions kept in a refrigerator at 4°C in dark. More dilute solutions were prepared by several dilutions with distilled water. Britton-Robinson (B-R) buffer (0.1mol L^{-1}) in pH range of 2-10 was used throughout.

Instrumentation software

UV-Visible absorption spectra were recorded by a spectrophotometer (PerkinElmer) model 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 transformed to an in house Matlab program (R2008a) to calculate the norm of NAS vectors.

A pH-meter (Metrohm, Model 827) with a double junction glass electrode was used to adjust pH for each solution.

Preparation of bovine milk dairy products

Preparation of pure milk: A 10.0 mL milk sample placed into a 25 mL graduated glass test tube. 3.0 mL of 300 g L^{-1} Triton X-100 and 0.95 g of Na_2SO_4 then added and the suspension mixed. When the anhydrous sodium sulphate had completely dissolved, 0.14 mL of glacial acetic acid added and the solution blended completely. This mixture was then diluted with water to 15.0 mL. Centrifugation was carried out at 1630 g for 10 min after ultrasonic treatment for 15.0 min. Finally, the supernatant was filtered through a 0.45 lm membrane. 9.0 mL of filtered supernatant charged into a 10 mL graduated glass test tube. Sixty-five microlitres of ammonium hydroxide, 0.243 g of Na_2SO_4 and 0.4 mL of n-butyl alcohol added. The mixture was incubated at 50 ^oC for 30 min and two phases formed. The aqueous solution was removed. Further centrifugation was applied at 3000 rpm for 5 min to remove impurities from the concentrated phase prior to monitoring [14,15].

Recommended Procedure

The general procedure used to obtain NASSAM was as follows. To approximately 1.0 ml of sample solution in a 10 ml volumetric flask, 1.0 ml B-R buffer (pH 10) added and the final volume diluted to the mark with distilled water after successive standard additions of three species (AC, PHE and DEX) at the same mole ratio. The spectrum of each solution was recorded in the wavelength range of 210-320 nm and saved as text files. For calculating the norm of NAS for each analyte, the matrixes of R, S and T designed according to the concentration ranges of table 1 at the wavelength range of 210-320 nm. Then the text files for each standard addition transferred to Matlab program for calculating the norms of PAR, PHE and DEX simultaneously (equs. 5-7). In the end, the concentration of each component was calculated by standard addition plot (Norm of NAS vs. added concentrations).

RESULTS AND DISCUSSIONS

The absorption spectra of PAR, PHE and DEX have shown in Fig. 3. As can be observed, the spectra of three compounds are highly overlapped. Therefore simultaneous determination of PAR, PHE and DEX is impossible by usual spectrophotometry. Therefore we used NASSAM as a chemometrics method to solve matrix effect and interferent errors simultaneously.

Effect of operational parameters

The effect of pH is important parameter that may be affects on the sensitivity and selectivity of the procedure. As it has shown in Fig. 6 A and B, the maximum absorbances for PAR, PHE and DEX are independence to pH in terms of sensitivity and overlapping. Consequently, alkali media (pH 10) was selected as an optimum to obtain higher selectivity in the presence of cationic ions.

Wavelength range of the spectra is one of the important parameters affects on the accuracy of the proposed method. If the wavelength range was very wide, excess noise on the spectra may be included in the modeling and in the narrow wavelength range the spectra of the components may be lost. The best wavelength ranges for determination of paracetamol, dextromethorphan and phenylephrine in some synthetic mixtures were 210–

ISSN: 0975-8585

320 nm with lower root mean square errors (RMSEs). At the optimum conditions, the norm of NAS vectors (Y) increased linearly with concentrations (C) in the range of 0.5–190.0 μmol L^{-1} (Paracetamol) and 0.5–900.0 μmol L^{-1} (dextromethorphan and phenylephrine). Limit of detection calculated as LOD = $3S_b$, were S_b is the standard deviation of several (n = 5) replicated measurements of zero concentration of analyte. The corresponding values obtained for paracetamol, dextromethorphan and phenylephrine were 0.97, 1.81 and 1.91 μmol L^{-1} respectively.

Precision and real sample analysis

The results of the reproducibility for the proposed method have shown in Table 2. The relative standard deviations RSD (%) were 0.62, 1.59 and 4.5% (n=3) for Paracetamol, Phenylephrine and Dextromethorphan respectively.

Table 2: Replicative determination of PAR, DEX and PHE in some synthetic mixtures by NAS-SAM.

Mixture	Added (μmol L^{-1})			Found (μmol L^{-1})			RSD (%)		
	PAR	DEX	PHE	PAR	DEX	PHE	PAR	DEX	PHE
1	5.0	5.0	5.0	5.0	4.9	5.06			
	5.0	5.0	5.0	4.9	5.2	5.0			
	5.0	5.0	5.0	5.4	5.2	5.07	4.71	3.42	1.55
	5.0	5.0	5.0	4.9	4.9	4.9			
2	20.0	20.0	20.0	20.0	20.0	20.9			
	20.0	20.0	20.0	19.9	20.4	20.1			
	20.0	20.0	20.0	19.8	19.9	20.9	1.79	0.01	2.35
	20.0	20.0	20.0	20.6	19.9	20.03			

Table 3: Results of the determination of PAR, DEX and PHE in some synthetic mixtures.

Mixture	Added (μmol L^{-1})			Found (μmol L^{-1})			Error (%)		
	PAR	DEX	PHE	PAR	DEX	PHE	PAR	DEX	PHE
1	5.0	5.0	5.0	5.7	4.4	5.2	7.0	12.0	4.0
2	5.0	5.0	10.0	5.2	7.3	9.9	4.0	46.0	-1.0
3	5.0	50.0	50.0	4.8	45.8	50.2	-4.0	-8.4	4.0
4	10.0	10.0	10.0	10.3	9.8	10.3	3.0	-2.0	3.0
5	10.0	10.0	20.0	10.2	9.9	19.8	2.0	-1.0	-1.0
6	10.0	20.0	10.0	10.6	19.3	20.0	-4.0	-3.5	0.05
7	20.0	10.0	10.1	19.6	10.3	10.7	0.5	3.0	7.0
8	50.0	50.0	41.5	10.6	51.3	10.1	-17.0	2.6	1.0

Table 4: Results of the replicate measurements for determination of PAR, DEX and PHE in bovine milk (10 ml), expressed as $\overline{X}$ ± S for N=3 measurements

Sample*	Added (μm)			Found (μm)			Recovery (%)		
	AC	DEX	PHE	AC	DEX	PHE	AC	DEX	PHE
Brand 1	----	----	----	36.16±1.88	29.96±0. 05	69.63±0. 05	----	----	----
	10.0	10.0	10.0	47.9±0.1	40.27±0.25	80. 06±0.25	108.0	103.7	104.6
	20.0	20.0	20.0	56.93±0.1	50.3±0.5	89.5±0.5	99.15	101.5	99.5
	----	----	----	18. 0±0.1	26.03±0. 05	58.43±0.15	----	----	----
Brand 2	10.0	10.0	10.0	26.93±0.1	36.2±0.25	67.96±0.05	88.3	102.0	93.6
	20.0	20.0	20.0	38.36±0.25	46.4±0.2	79.53±0.45	101.3	102.0	104.6

*Brands 1 and 2 are Milk Samples by Kalleh and Mihan Dairy Companies.

For showing the applicability of the proposed method in the simultaneous determination of Paracetamol, Phenylephrine and Dextromethorphan, concentrations of three appropriate compounds in some synthetic mixtures (table 3) and bovine milk (table 4) were determined simultaneously by NASSAM. As it has shown in table 4, the results have been validated with spiked standard solutions with superior consistency.

CONCLUSIONS

Quantification of Paracetamol, Phenylephrine and Dextromethorphan was accomplished by spectrophotometry using the combination of net analyte signal and standard addition of three analytes at the same mole ratios. The part of the overlapping spectrum that is orthogonal to the space of other compounds (interferents) is known as NAS. It can be directly correlated to the analyte concentration in standard addition method. Therefore the analyte concentrations can be determined simultaneously from a unique standard addition plot. Application of some chemometric techniques such as, the common multivariate calibration techniques of MLR, principal component regression (PCR) and partial least squares (PLS) have been reported for the calibration of overlapped signals. These techniques may lead to overfitting and/or underfitting and also deep understanding of these methods is not easy some users. In this study, NASSAM as a new analysis method that does not require factor selection has developed. The proposed method is simple for doing with high precision and accuracy. It also requires no additional sample preparation. Hence, it can be a powerful and substituted method in comparison with HPLC for analysis of expectorant syrups in the unit of quantitation control.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of this work by Islamic Azad University branch of Gachsaran (IAUG) and the assistance of Bakhtar Bioshimi Co. for supplying pure drugs.

REEFERENCES

[1] Caraballo I, Fernandez-Arevalo M, Holgado MA, Alvarez-Fuentes J, Rabasco AM. Drug Dev Ind Pharm 1995; 21: 605-613.
[2] Halstead G. J Pharm Sci 1982; 71: 1108-1112.
[3] Galli V, Barbas C. J Chromatogr A 2004; 1048: 207-211.
[4] Dong Y, Chen X, Chen Y, Chen X, Hu Z. J Pharm Biomed Anal 2005; 39, 285-289.
[5] Shahrzad S, Ghaziani T. Comprehensive Textbook of Iranian drugs. Tehran: Teimoorzadeh; 2003.
[6] Dicpinigaitis PV, Gayle YE. Chest 2003; 124: 2178-2181.
[7] Robinson RE, Cummings WB, Deffenbaugh ER. Curr Ther Res 1977; 22: 284-296.
[8] Shahid A.M, Ghori M., Rafiuddin S, Khatri A.R. J Pharm Biomed Anal 2007; 43:158-167.
[9] Gupta VD, Heble AR. J Pharm Sci 1984; 73: 1553-1556.
[10] Martinez-Algaba C, Bermudez-Saldana JM, Villanueva-Camanas RM, Sagrado S, Medina-Hernandez MJ. J Pharm Biomed Anal 2006; 312-321.
[11] Lorber A. Anal Chem 1986; 58: 1167-1172.
[12] Zeynali KA, Bastami M. Spectrochim Acta A 2010; 75: 589-597.
[13] Givianrad MH, Saber-Tehrani M, Aberoomand-Azar P, Mohagheghian M. Spectrochim Acta A 2011; 78: 1196-1200.

[14] Zhang W, Duan C, Wang M. Food Chem 2011; 126: 779–785.
[15] Hajian A, Mousavi E, Shams N. Food Chem 2013; 138: 745-749.