

Simultaneous Determination of Hydrochlorothiazide and Enalapril Maleate in Pharmaceutical Formulations Using Fourier Transform Infrared Spectrometry

Ahmadi, Seyyed Hamid

Chemistry and Chemical Engineering Research Center of Iran, Tehran, I.R. IRAN

Tavakoli, Hassan; Amirzadeh, Majid*⁺

Department of Chemistry, Emam Ali University, Tehran, I.R. IRAN

Sangi, Mohammad Reza

Department of Chemistry, Arak University, Arak, I.R. IRAN

ABSTRACT: A new Fourier Transform-Infra Red (FT-IR) spectrometric method was developed for assaying hydrochlorothiazide (HCT) and enalapril maleate (ENM) in binary solid pharmaceutical formulations. Multivariate Partial Least Squares (PLS) method was used for calibration of derivative spectral data. Acetonitrile was used as solvent due to its spectral transparency and adequate solubility of analytes in it. A 4-levels full factorial design of binary standard solutions of HCT and ENM were prepared and used for calibration in the spectral range of 1550-1800 cm^{-1} . Statistical parameters such as correlation coefficient (R), Standard Error of Estimation (SEE), Standard Error of Prediction (SEP), and Standard Error of Cross Validation (SECV) have been evaluated and used for selecting of optimizing of parameters. Correlation coefficients were 0.9990 and 0.9995 and Relative Standard Deviation (RSD) were 1.97% and 1.35% ($n=5$) for HCT and ENM, respectively. Detection limits of ENM and HCT were obtained 0.54 and 0.99 mg/mL respectively. The proposed methods were successfully applied to the determination of the over mentioned drugs in laboratory-prepared mixtures and in commercial tablets. This method has suitable accuracy, precision, repeatability and is comparable with reference standard methods.

KEY WORDS: FT-IR; Pharmaceutical formulation; Simultaneous determination; Quantitative analysis, Hydrochlorothiazide, Enalapril maleate.

INTRODUCTION

Enalapril maleate (ENM), {N-[(S)-1-ethoxycarbonyl-3-phenyl propyl]-L-alanyl}-L-proline hydrogen maleate (1) (Fig. 1), is an angiotensin-converting enzyme inhibitor

used in the treatment of hypertension and heart failure. It is also used to reduce the incidence of coronary ischemic events, including myocardial infarction (1).

* To whom correspondence should be addressed.

+ E-mail: majidaz23@yahoo.com

1021-9986/14/4/59

9/\$/2.90

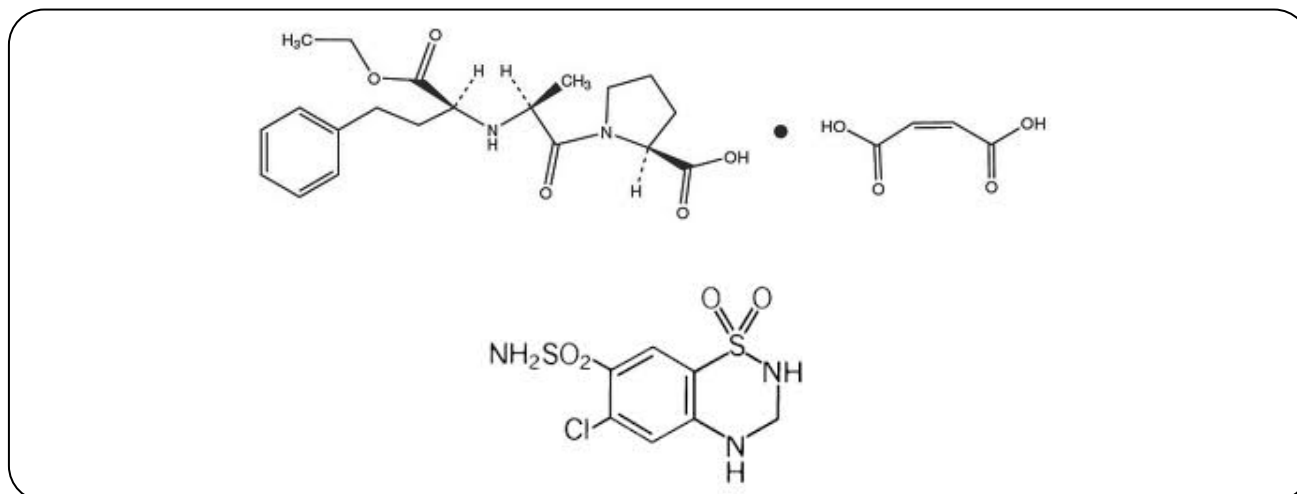


Fig.1: Structural formula of (top) ENM and (bottom) HCT.

Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide [1] (Fig. 1), is a diuretic of benzothiadiazine class, extremely useful in the treatment of edema, hypertension and hypercalciuria [2]. A new combination dosage form containing ENM and HCT is indicated for the treatment and management of edema and hypertension.

A literature review revealed that several methods have been described for the determination of ENM in pure forms and in pharmaceutical preparations including spectrophotometry [3-5], spectrofluorimetry [6], capillary electrophoresis [7] and High Performance Liquid Chromatography (HPLC) [8-10]. Regarding HCT, various methods have been reported for its determination, such as spectrophotometry [11, 12], derivative spectrophotometry [13-15], HPLC [16, 17] and liquid Chromatography-Mass Spectrometry (LC-MS) [18, 19]. Different methods were reported in the literature for the determination of ENM and HCT in their co-formulated tablets relied on spectrophotometric methods [20], derivative spectrophotometric method [21, 22], capillary electrophoresis [23], High Performance Thin Layer Chromatography (HPTLC) [24], HPLC methods for their determination, either alone or in the presence of other cardiovascular agents [25, 26] and Ultra Performance Liquid Chromatography (UPLC) [27].

All these techniques require lengthy labor intensive procedures which could not be ideal for routine analyses in quality control laboratories. FT-IR spectroscopy is a simple, rapid and non-destructive technique which

is playing a great role for the rapid determination of various components present in the simple as well as complex matrices [28-30]. Initially FT-IR Spectroscopy was considered as a qualitative tool but since last two decades tremendous quantitative work has been published. Thus FTIR spectroscopy along with chemometrics can be applied efficiently from raw material identification to final product analysis of pharmaceutical formulations. Non-destructive and fast analytical nature of FT-IR spectroscopy can be exploited in an advantageous manner in wide range of applications in modern industries. In spectral analysis quantitative calibration techniques like chemometrics has been regarded as an important tool while monitoring quality and quantity of drugs in pharmaceutical formulations or biological systems, as it provides the advantage of not requiring any separation procedure in analysis of two or more drugs having spectral overlapping [31, 32].

The enalapril maleate-hydrochlorothiazide mixture is not yet official in any pharmacopoeia. To our knowledge, no infrared spectrometric methods have been described for the simultaneous determination of both drugs in tablets. Therefore, it is desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the determination of both drugs in the presence of each other. In this report, a FT-IR based method is investigated for the quantification of both drugs in their solid dosages. The utility of developed method to determination the content of both drugs in commercial tablets is also demonstrated.

EXPERIMENTAL SECTION

Apparatus

A Vector 22 FT-IR spectrometer from Bruker (Ettlingen, Germany) equipped with a DTGS mid-range detector, a Ge/Sb2S3-coated KBr beam splitter and a Global source was employed for spectral measurements. Version 4 of Opus software developed by Bruker, was employed for spectral measurements. Omnic 1.2 and Quant IR 1.2 software packages from Nicolet(Madison, USA) were used for acquisition of the spectra, statistical treatment of data and performing the PLS method, respectively. Infrared spectra were obtained by accumulating 32 scans at a resolution of 4 cm⁻¹. A transmission flow through cell, which was assembled using zinc selenide (ZnSe) windows and a 0.5 mm Teflon spacer in cell body(b=0.5mm), was used for experiments.

Reagents and samples

Authentic enalapril maleate and hydrochlorothiazide were used as purchased. The purity of both drugs as assessed by the USP XXII methods was 98.5% and 99.1% for enalapril maleate and hydrochlorothiazide, respectively. All solvents and reagents were of analytical grade and used without further purification. An orthogonal design with a training set of standards containing ENM and HCT at four concentration levels covering a total of 16 standard solutions, based on the model 4² standard was taken [34]. The compositions of these binary mixtures used in the calibration matrix design are summarized in Table 1. Seven more binary standard solutions were prepared for evaluation of this calibration matrix design by mixing different weights of two calibration samples. The composition of these two validation set is also shown in Table 1. Each standard sample was prepared by dissolution of accurately weighted amounts of ENM and HCT in the suitable solvent.

Procedure

Each sample was injected into the cell by a glass syringe. IR spectra were collected at a nominal resolution of 4 cm⁻¹ with 32 scans. The spectrum of solvent was applied as background. The pharmaceutical samples were accurately weighed and grinded in mortar until fine powder was obtained. Accurately weighted amounts

Table 1: Concentration data for binary standard solutions used as calibration set (1-16) and validation set (17-23).

Standard solution	Concentration, mg/mL	
	ENM	HCT
1	0.00	0.00
2	2.00	0.00
3	5.00	0.00
4	7.00	0.00
5	0.00	3.00
6	2.00	3.00
7	5.00	3.00
8	7.00	3.00
9	0.00	5.00
10	2.00	5.00
11	5.00	5.00
12	7.00	5.00
13	0.00	8.00
14	2.00	8.00
15	5.00	8.00
16	7.00	8.00
17	6.00	5.50
18	4.50	5.50
19	3.50	4.25
20	3.75	4.75
21	3.25	4.50
22	6.00	6.50
23	4.50	5.00

of the powders were dissolved in the solvent and the prepared solutions were injected to the cell for spectral recording.

RESULTS AND DISCUSSION

Selection of Solvent and Spectral region

Acetonitrile was selected as best choice for the dissolution of both drugs due to its relative spectral transparency in selected spectral range, good solubilities of ENM and HCT in it and negligible spectral interferences of drug excipients. Fig. 2 shows the absorbance spectra of HCT and ENM in wavenumber range of 4000 – 500 cm^{-1} in solid form (as KBr disk) in comparison with the absorption spectrum of solvent acetonitrile.

Acetonitrile shows relative transparencies in 1900-1500 cm^{-1} range which included several intense absorption bands of ENM and HCT. Therefore the over mentioned spectral regions were used for the calibration study.

PLS Calibration Model

Fig. 3 shows IR spectra of standard solutions in selected spectral region after acquiring FTIR spectra of the mixture of the drugs; PLS model was applied to establish calibration for the quantitative determination of ENM and HCT in pharmaceutical formulations. The region of 1800 - 1550 cm^{-1} for was selected from the same spectra corrected with two point baseline identical to the region. The remarkable correlation coefficients (R^2) were achieved 0.9948 and 0.9974 respectively between actual and predicted values with small values of Standard Error of Estimation (SEE), Standard Error of Cross Validation (SECV) and Standard Error of Prediction (SEP) 0.43, 1.05 and 1.02 for HCT and 0.33, 0.00645 and 0.93 for ENM, respectively. The good calibration curve between the actual and calculated values for calibration standards is obtained. It is clearly evident that the calibrated model has predicted concentration of analytes well as calculated values are scattered within a narrow concentration range of 2% to 4% as compared with the original concentration.

The problem of overlapping spectra of ENM and HCT has also been circumvented by making use of their derivative spectra. Fig. 4 shows the first derivative spectra of ENM and HCT and their binary mixtures

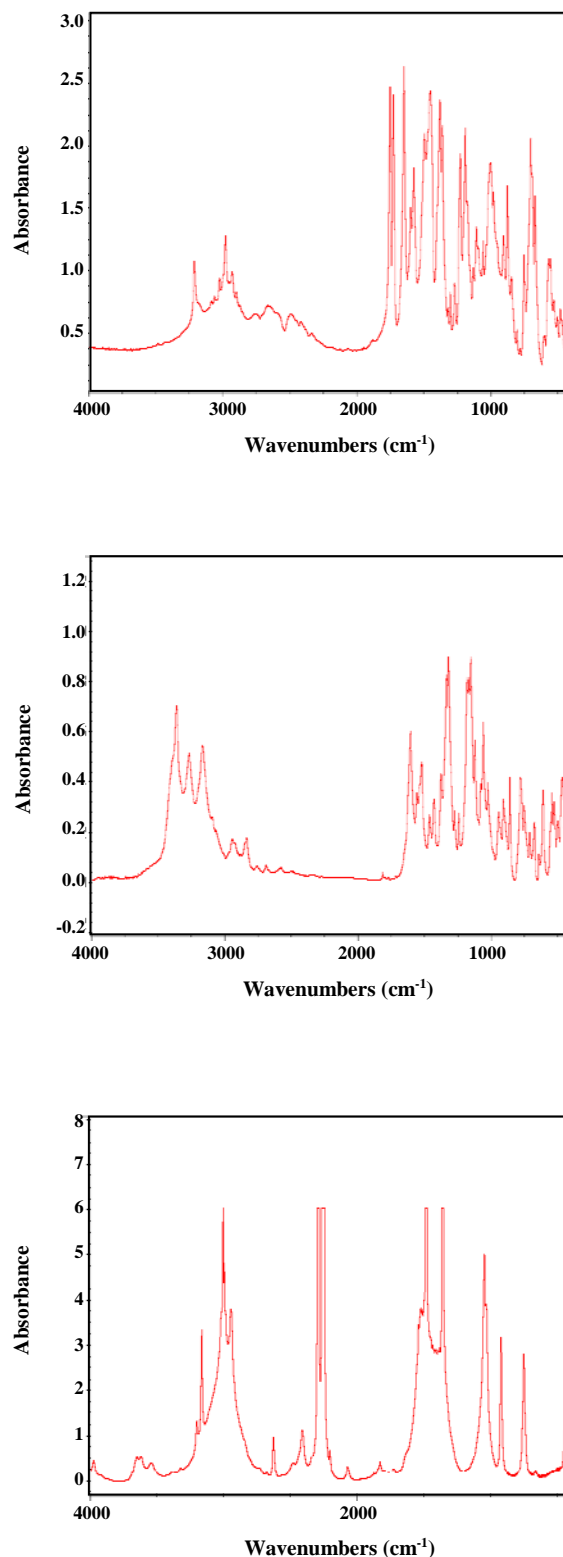


Fig.2: The IR absorption spectra of A: HCT, B: ENM as KBr disks and C: Acetonitrile as thin liquid film.

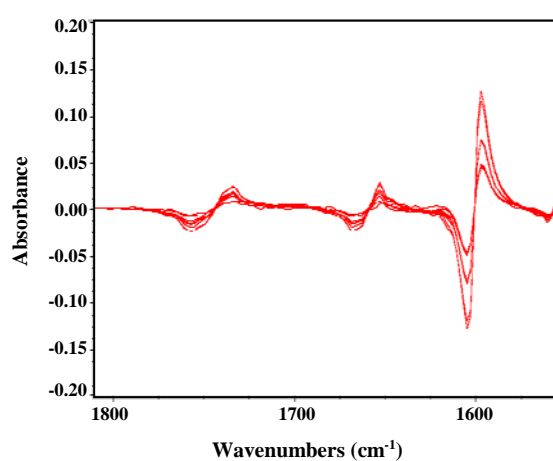
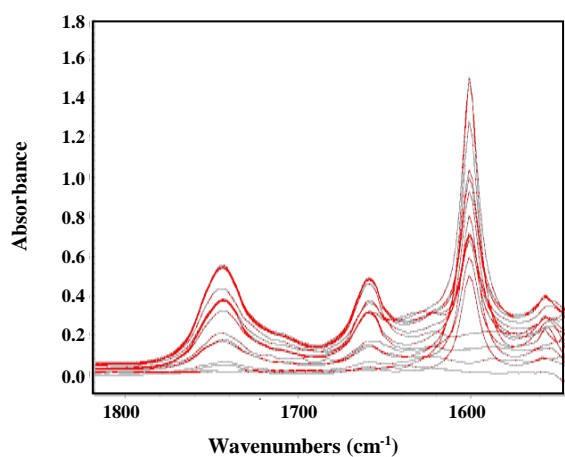
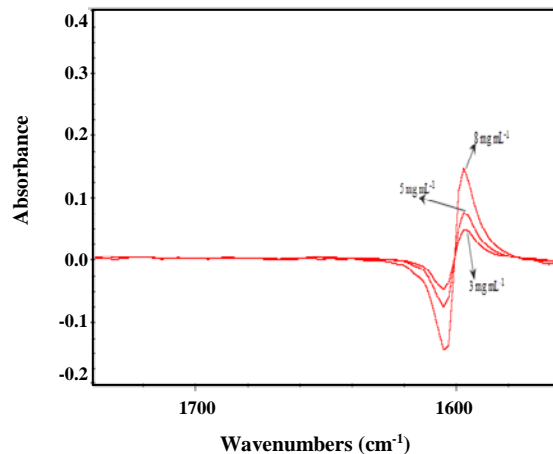
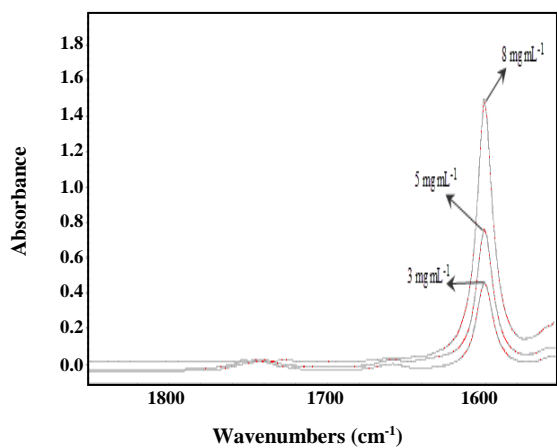
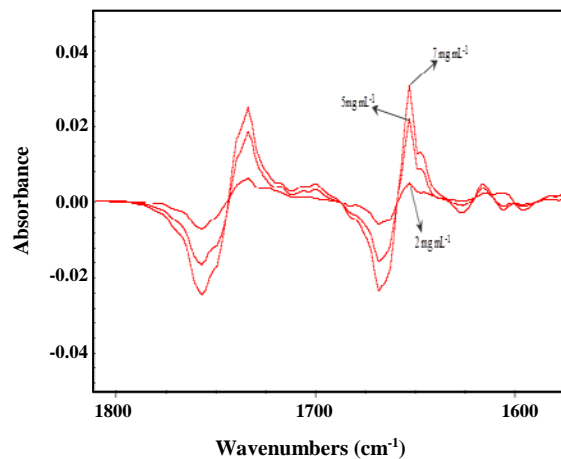
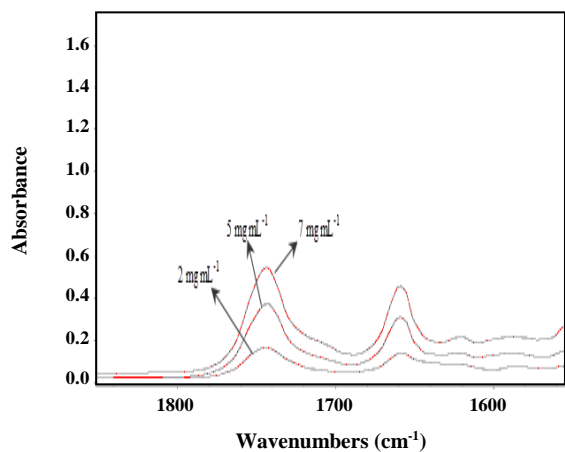


Fig. 3: IR absorption spectra of standard solutions containing different concentrations of A: ENM (2, 5, 7 mg / mL), B: HCT (3, 5, 8 mg / mL) and C: their nine binary solutions (overmentioned concentrations).

Fig. 4: IR derivative spectra of standard solutions containing different concentrations of A: ENM (2, 5, 7 mg / mL), B: HCT (3, 5, 8 mg / mL) and C: their nine binary solutions (with overmentioned concentrations) in the spectral region of 1550-1850 cm^{-1} .

Table 2 Comparison of results obtained in analyzing samples of calibration and validation sets at two PLS methods in calibration of spectra data at region of 1850-1550 cm⁻¹.

Statistical parameter	R ²		SEE*		SECV*		SEP*		Optimum factor number	
	HCT	ENM	HCT	ENM	HCT	ENM	HCT	ENM	HCT	ENM
Absorption spectra	0.9948	0.9974	0.43	0.33	1.02	0.93	1.05	1.02	2	2
Derivative spectra	0.9990	0.9995	0.33	0.18	0.76	0.36	0.64	0.35	2	2

*: in mg/mL

at various concentration levels. These derivative spectra were also applied for PLS calibration using two-point baseline correction. The viability of two PLS models were tested by comparing the optimal number of factors, R, SEE, SECV and SEP obtained for each component of binary standard samples in each model. To select the optimum number of factors in the PLS algorithm in order to model the system without overfitting the concentration data, a cross validation method,⁸ leaving out one sample at a time, was used for both sets of standards^(32, 35). The predicted concentrations of the compounds in the sample left were compared with the actual concentrations in this reference sample and the Prediction Error Sum of Squares (PRESS) was calculated:

$$\text{PRESS} = \sum_{i=1}^n (C_i - C'_i)^2$$

Where n is the number of standards, C_i is the actual concentration of analyte in the sample i and C'_i represents the predicted concentration of the analyte in the sample i. One reasonable choice for the optimum number of factors (h*) would be that number which yielded the minimum PRESS; however, this sometimes leads to overfittings. A better criterion is to select the model with the fewest number of factors such that PRESS for that model is not significantly greater than PRESS from the model with h* factors. The F-statistic was used for the significance determination. Haaland and Thomas empirically determined that an F-ratio probability of 0.75 is a good choice [36]. We selected as optimum the number of factors for the first PRESS value the F-ratio probability of which drops below 0.75.

The optimal number of factors, SEE, SECV, SEP and R² values obtained for PLS calibration of absorption and first derivative spectral data of HCT and ENM in calibration and validation sets are summarized in Table 2. R² (correlation coefficient square) values of PLS calibration

of derivative spectral data are better than calibration of absorption spectral data for both ENM and HCT. Smaller standard error values of derivative PLS method in comparison with first method, confirm same result.

Precision and validation of selected method were also studied by prediction of content of each analyte in a set of artificial binary dosages which prepared by addition of various amounts of ENM and HCT to 200 mg synthetic Tablet 1 matrix (containing suitable amounts of corn starch, lactose, calcium phosphate and magnesium stearate as excipients of solid formulations). Table 3 shows composition of each sample of this independent standard validation set. Each sample was leached with 4 mL acetonitrile, and the leached solution was injected to cell for spectrometric experiment after filtration (for removing undissolved materials). Replicate analyses of first standard showed relative standard deviation values of 1.35% and 1.97% for ENM and HCT, respectively (n=5). Good correlation of added and found amounts shows considerable appropriation for FTIR simultaneous determination of ENM and HCT in tablet dosages using derivative spectra and PLS calibration.

Application

The proposed method was tested to determine ENM and HCT in commercial tablets. Vaseretic[®] tablets from Valeant Pharmaceuticals International Inc. (Steinbach, Canada) and Envas-H[®] tablets from Cadila pharmaceuticals Ltd. (New Delhi, India) were purchased from local drug stores for this purpose. Ten tablets were weighed accurately and after grinding by mortar and pestle, the powder was mixed thoroughly quantity of powder equivalent to one tablet was dissolved in 4 mL of acetonitrile and after filtering, introduced to flow cell for the analysis.

In the analysis of the commercial, the found amounts and recoveries were achieved by comparing with claimed amounts. An ultraviolet spectrometric method was also

Table 3: The results of selected method in prediction of standard addition samples.

sample	Added(mg)		Found(mg)	
	ENM	HCT	ENM	HCT
1*	8.00	12.00	7.71	12.23
2	16.00	12.00	16.08	12.20
3	20.00	12.00	19.13	12.28
4	23.75	12.00	22.46	12.73
5	8.00	22.00	7.95	21.65
6	8.00	27.00	7.66	26.48
7	8.00	30.00	7.68	29.76

*: The sample was tested for precision study (n=5)

Table 4: Determination of ENM and HCT in two commercial Tablets (n=5)

Amounts(mg)	Vasertic®		Envas-H®	
	ENM	HCT	ENM	HCT
Claimed	10	25	5	12.5
Proposed method	10.03±0.12	25.13±0.23	4.57±0.11	12.13±0.17
Reference Method	10.39±0.15	26.56±0.33	4.56±0.23	12.56±0.27

performed as reference method on the commercial tablets. Table 4 Shows the results of the assay of pharmaceutical tablets by proposed and reference method [21].

CONCLUSIONS

This work proves that the application of FT-IR spectrometry to overdetermined multicomponent systems allows determinations to be carried out in difficult cases when the extreme spectral overlap would prohibit determinations based on zero-order spectra. Calibration of first-order IR spectra was performed using partial least squares model. Multivariate methods present clear advantages over univariate ones as they allow the detection of second-order effects.

The proposed method shows considerable appropriation for the simultaneous determination of enalapril maleate and hydrochlorothiazide in commercial tablets. Small amounts of samples are employed with minimum treatment and the spectra are collected rapidly. The accuracy and precision in proposed method can be similar and possibly better than those obtained by referenced methods, such as HPLC. The presented method is more fast, safe, simple and lower-cost in comparison with liquid chromatographic methods in routine analyses of ENM and in pharmaceutical quality

control laboratory labs. These results show that the method proposed is effective and suitable for the simultaneous determination of ENM and HCT in pharmaceuticals.

Received : Dec. 14, 2013 ; Accepted : Aug. 25, 2014

REFERENCES

- [1] Sweetman S.C. (ed), "Martindale: the Complete Drug Reference", 35th ed., The Pharmaceutical Press, London, (2007).
- [2] Block J., Beal J.M. (eds), "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 11th ed., Lippincott Williams & Wilkins, Philadelphia, (2004).
- [3] Patil P.S., More N., Difference Spectrophotometric Estimation of Enalapril Maleate from Tablet Dosage Form, *Internat. J. Res. Pharmac. Biomed. Sci.*, **2**: 629-633 (2011).
- [4] Dubey S., Kum S., Mudakavi R.J., Deshpande S., Development and Validation of UV-Spectrophotometric Method for Determination of Enalapril Maleate, *International Journal of Advance Pharmaceutical Science*, **1**: 375- 386 (2010).

- [5] Rahman N., Manirul Haque S.K., [Optimized and Validated Spectrophotometric Methods for the Determination of Enalapril Maleate in Commercial Dosage Forms](#), *Analytical Chemical Insights*, **3**: 31-43 (2008).
- [6] Oliva M.D.A., Sombra L.L., Olsinal R.A., Masi A.N., [A New Fluorescent Assay for Enalapril Maleate](#), *Journal of Fluorescence*, **15**: 723-735 (2005).
- [7] Hillaert S., Van-Den-Bossche W., [The Quantitative Determination of Several Inhibitors of the Angiotensin-Converting Enzyme by CE](#), *Journal of Pharmaceutical and Biomedical Analysis*, **25**: 775-783 (2001).
- [8] Bharat G.C., [Development and Validation of RP-HPLC Method for Simultaneous Estimation of Enalapril Maleate and Amlodipine Besylate in Combined Dosage Form](#), *Applied Pharmaceutical Science*, **2**: 54-69 (2012).
- [9] Dinc E., Uzdemir A., Baleanu D.J.A., [New Application of Chemometric Techniques to HPLC Data for the Simultaneous Analysis of a Two-Component Mixture](#), *Liquid Chromatography and Related Technologies*, **28**: 2179-2194 (2005).
- [10] Pisarev V.V., Moskaleva N.E., Zverkov Y.B., Smirnova L.B., Belolipetskaya V.G., Sukhanov Y.V., [HPLC/MS Determination of Enalapril and Enalaprilat in the Blood Plasma](#), *Journal of Pharmaceutical Chemistry*, **39**: 104-107 (2005).
- [11] [The British Pharmacopoeia](#), The Stationery Office, London, (Electronic version) (2010).
- [12] Omar M.A., [Spectrophotometric and Spectrofluorimetric Determination of Certain Diuretics through Ternary Complex Formation with Eosin and Lead \(II\)](#), *Journal of Fluorescence*, **20**: 275-281 (2010).
- [13] Shah D.A., Bhatt K.K., Mehta R.S., Baldania S.L., [Determination of Nebivolol Hydrochloride and Hydrochlorothiazide in Tablets by Firstorder Derivative Spectrophotometry and Liquid Chromatography](#), *Journal of AOAC International*, **91**: 1075-1088 (2008).
- [14] Stolarczy K.M., Apola A., Krzek J., Lech K., [Simultaneous Determination of Triamterene and Hydrochlorothiazide in Tablets Using Derivative Spectrophotometry](#), *Acta Poloniae Pharmaceutica*, **65**: 283-298 (2008).
- [15] Mohammadpour K., Sohrabi M.R., Jourabchi A., [Continuous Wavelet and Derivative Transform Applied to the Overlapping Spectra for the Quantitative Spectrophotometric Multi-Resolution of Triamterene and Hydrochlorothiazide in Their Tablets](#), *Talanta*, **81**: 1821-1836 (2010).
- [16] Vijayasree V., Pallavan C., Seshagiri Rao J.V., [Development and Validation of an RP-HPLC Method for the Estimation of Hydrochlorothiazide in Tablet Dosage Forms](#), *International Journal of Pharmaceutical Sciences and Research*, **4**: 1052-1066 (2013).
- [17] Rane V.P., Sangshetti J.N., Shinde D.B., [Simultaneous High Performance Liquid Chromatographic Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Preparation](#), *Journal of Chromatographic Sciences*, **46**: 887-892 (2008).
- [18] Yan T., Li H., Deng L., Guo Y., Yu W., Fawcett J.P., [Liquid Chromatographic-Tandem Mass Spectrometric Method for the Simultaneous Quantitation of Telmisartan and Hydrochlorothiazide in Human Plasma](#), *Journal of Pharmaceutical and Biomedical Analysis*, **48**: 1225-1233 (2008).
- [19] Kumar V., Shah R.P., Singh S., [LC and LC-MS Methods for the Investigation of Polypills for the Treatment of Cardiovascular Diseases. Part 1. Separation of Active Components and Classification of Their Interaction/ Degradation Products](#), *Journal of Pharmaceutical and Biomedical Analysis*, **47**: 508-522 (2008).
- [20] Sowjanya G., Gangadhar P., Ramalingeswara Rao P., Subrahmanyam P., Suresh P., [Simultaneous UV Spectrophotometric Estimation of Enalapril Maleate and Hydrochlorothiazide in Tablets](#), *Journal of Chemical and Pharmaceutical Research*, **4**: 3483-3495 (2012).
- [21] Mariusz S., Anna M., Jan K., Joanna M., [Application of Derivative Spectrophotometry for Determination of Enalapril, Hydrochlorothiazide and Walsartan in Complex Pharmaceutical Preparations](#), *Acta Poloniae Pharmaceutica*, **65**: 275-283 (2008).
- [22] Walily E.I., Belal A.F., Heaba S.F., El E.A., Kersh A.A., [Simultaneous Determination of Enalapril Maleate and Hydrochlorothiazide by First Derivative Ultraviolet Spectrophotometry and High-Performance Liquid Chromatography](#), *Journal of Pharmaceutical and Biomedical Analysis*, **13**: 851-866 (1995).

- [23] Hillaert S., De Grauwe K., Van den Bossche W., Simultaneous Determination of Hydrochlorothiazide and Several Inhibitors of Angiotensin-Converting Enzyme by Capillary Electrophoresis, *Journal of Chromatography A*, **924**: 439-446 (2001).
- [24] Kondawar M., Gaikwad R., Apaté V., Ravetkar A., High Performance Thin Layer Chromatographic Determination of Enalapril Maleate and Hydrochlorothiazide in Pharmaceutical Dosage Form, *International Journal of PharmTech Research*, **3**: 1454-1464 (2011).
- [25] Elsebaei F., Zhu Y., Fast Gradient High Performance Liquid Chromatography Method with UV Detection for Simultaneous Determination of seven Angiotensin Converting Enzyme Inhibitors Together with Hydrochlorothiazide in Pharmaceutical Dosage Forms and Spiked Human Plasma and Urine, *Talanta*, **85**: 123-133 (2011).
- [26] Gonzalez O., Iriarte, G., Rico E., Ferreiro N., Maguregui M.I., Alonso R.M., LC-MS/MS Method for the Determination of Several Drugs Used in Combined Cardiovascular Therapy in Human Plasma, *Journal of Chromatography B*, **878**: 2685-2693 (2010).
- [27] Uslu B., Ozden T., HPLC and UPLC Methods for the Simultaneous Determination of Enalapril and Hydrochlorothiazide in Pharmaceutical Dosage Forms, *Chromatographia*, **76**: 1487-1498 (2013).
- [28] Masoum S., Alishahi A.R., Farahmand H., Shekarchi M., Prieto N., Determination of Protein and Moisture in Fishmeal by Near-Infrared Reflectance Spectroscopy and Multivariate Regression Based on Partial Least Squares, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **31**(3): 51-59 (2012).
- [29] Khaskheli M.A., Sherazi S.T.H., Ujan H.M., Mahesar S.A., Transmission FT-IR Spectroscopic Analysis of Human Kidney Stones, *Turkish Journal of Chemistry*, **36**(3): 477-483 (2012).
- [30] Mahesar S.A., Sherazi S.T.H., Kandhro A.A., Bhangar M.I., Khaskheli A.R., Talpur M.Y., Evaluation of Important Fatty Acid Ratios in Poultry Feed Lipids by ATR FTIR Spectroscopy, *Vibrational Spectroscopy*, **57**(2): 177-187 (2011).
- [31] Sherazi S.T.H., Ali M., Mahesar M.A., Application of Fourier-Transform Infrared (FT-IR) Transmission Spectroscopy for the Estimation of Roxithromycin in Pharmaceutical Formulation, *Vibrational Spectroscopy*, **55**(1): 115-121 (2011).
- [32] Kargosha K., Ahmadi S.H., Ghassempour A., Arshadi M.R., Simultaneous Determination of the Pesticide Naptalam and Its Metabolites in Natural Water by Fourier Transform Infrared Spectrometry, *Analyst*, **124**: 367-376 (1999).
- [33] Kargosha K., Ahmadi S.H., Simultaneous Determination of Sulphamethoxazole and Trimethoprim in Co-Trimoxazole Tablets by First Derivative FTIR Spectrometry, *Anal. Lett.*, **32**(8): 1613-1619 (1999).
- [34] Harvey D., "Modern Analytical Chemistry", McGraw-Hill, New York, (2000).
- [35] Asadi S., Gharbani P., Simultaneous Determination of Sulfamethoxazole and Phthalazine by HPLC and Multivariate Calibration Methods, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **32**(2): 1-15 (2013).
- [36] Haaland D.M., Thomas E.V., Partial Least-Squares Methods for Spectral Analyses. 1. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information, *Anal. Chem.*, **60**(11): 1193-1202 (1988).