Rev Anal Chem 31 (2012): 107–112 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/revac-2011-0013

Using derivative and ratio spectra derivative: spectrophotometries as new data preprocessing method in partial least squares technique for resolving overlapped spectra of montelukast sodium, desloratadine, and levocetirizine dihydrochloride

Ismail Murat Palabiyik*, Mehmet Gokhan Caglayan and Feyyaz Onur

Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, Tandogan, Ankara 06100, Turkey, e-mail: mpala@pharmacy.ankara.edu.tr

*Corresponding author

Abstract

Chemometric techniques are widely used for resolving overlapped spectra in different analysis type including simultaneous determination of active ingredients in pharmaceutical preparation. Processed data are used in some instances alternative to the raw data. Standardization, normalization, and mean centering are preferred data preprocessing methods in multivariate analysis techniques. In this study, derivative and ratio spectra derivative spectrophotometric methods are used in preprocessing for spectrophotometric data as an alternative to the other procedure in partial least squares technique (PLS-1) for resolving overlapped spectra of ternary mixtures of montelukast sodium, desloratadine, and levocetirizine dihydrochloride. Simultaneous determination and quantitation of these active ingredients from their bulk solutions and pharmaceutical preparations was achieved successfully by this developed and optimized partial least squares method. These results indicate that derivative and ratio spectra derivative spectrophotometric methods will be used as a new data preprocessing method in chemometric techniques from now on.

Keywords: data preprocessing; derivative spectrophotometry; partial least squares; pharmaceutical analysis; ratio derivative spectrophotometry.

Introduction

Chemometric techniques are used extensively for analyzing of data obtained from different analytical equipment in a wide range of applications. These multivariate applications are powerful techniques due to easy adaptation of mathematical and statistical program to the traditional analytical techniques. Owing to this advantage, these are also used in pharmaceutical analysis especially in resolving overlapped spectra of active ingredients in their pharmaceutical preparations and optimization of the experimental conditions in that type of analysis.

Data preprocessing is a part of chemometric techniques, and in some cases, it is preferred for evaluation of analytical data. Standardization, normalization, and mean centering are classical methods for this purpose. These are calculated by any software program like Excel by Microsoft or, sometimes, are the parts of chemometric software program. Graphical procedures of spectra, such as derivative and ratio spectra derivatives, are the mathematical process for simultaneous determination of samples from different sources like pharmaceutical preparations via resolving of overlapped spectrum. Helping with these, simultaneous analysis of active ingredients from their mixture, without any chemical pretreatment and interference effects, is achieved in spectrophotometric analysis, namely, derivative and ratio spectra derivative spectrophotometric methods. Because these processes are used for evaluation of data, these will be applicable as a preprocessing method in chemometric techniques. Until now, there have not been any studies about that in the literature.

Montelukast sodium (MON), 1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2quinolinyl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropanacetic acid sodium (Figure 1A) is a leukotriene inhibitor used in seasonal allergic rhinitis (Philip et al. 2002, Topuz and Ogmen 2003). Desloratadine (DES), 8-chloro-6,11-dihydro-11-(4-piperidinylidine)-5 H-Benzol[5,6] cyclohepta [1,2-b] pyridine (Figure 1B), is a tricylic antihistamine and has a H1 receptor antagonist action. It reduces nasal congestion, hay fever, itching of eyes, and nasal flow in allergic patients (Nayak and Schenkel 2001). Levocetirizine dihydrochloride (LEV), 2-[2-[4-[(R)-(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride (Figure 1C), is the L-enantiomer of cetirizine and has twice the affinity for H1 receptors as cetirizine. It is also used in the treatment of hay fever (Aronson 2006). Binary combinations of MON and DES and MON and LEV are widely used in the treatment of the symptoms of allergic rhinitis. Simultaneous determination of MON and LEV was achieved by high-performance liquid chromatography and high-performance thin layer chromatography and first-

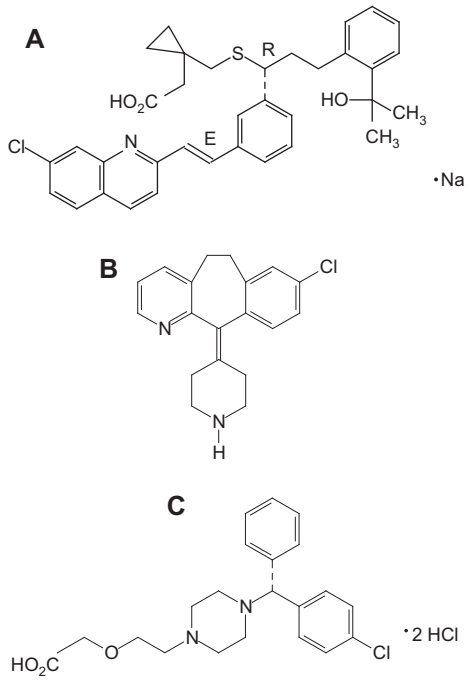

Figure 1 Chemical structure of (A) montelukast sodium, (B) desloratadine, and (C) levocetirizine HCl.

derivative spectrophotometry (Rote and Niphade 2010, 2011). There is also a study about simultaneous determination of MON and DES using high-performance liquid chromatography (Dhandayuthan et al. 2011).

The aim of this study was to develop a partial least squares (PLS-1) method as a chemometric technique for the simultaneous determination of MON, DES and LEV in one training set alternative to high-performance liquid chromatographic method as a separation technique. Derivative and ratio spectra derivative spectrophotometries were used as a new data preprocessing technique besides mean centering and standardization. These optimized and validated methods were successfully applied to two different pharmaceutical applications containing these active ingredients, and obtained results were compared for all of the applied data preprocessing techniques using Student's t-test and F-test.

Experimental

Reagents and chemicals

Montelukast sodium (100.3%, w/w) (Matrix Laboratories Limited, Secunderabad, India), levocetirizine dihydrochloride (98.8%, w/w) (Dr. Reddy, Andhra Pradesh, India), and desloratadine (FARGEM, Duzce, Turkey) were used without further purification. Methanol was purchased from Merck, Darmstadt, Germany.

DESMONT® (5 mg desloratadine and 10 mg montelukast sodium/film-coated tablet, batch no: 01090) (Nuvomed, Istanbul, Turkey) and LEVMONT® (5 mg levocetirizine hydrochloride and 10 mg montelukast sodium/film-coated tablet, Batch No: 01060) (Nuvomed, Istanbul, Turkey) were used as pharmaceutical preparations.

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and standard quartz cuvette (10 mm) was used for the spectrophotometric measurements. A heating magnetic stirrer (Velp Scientifica, ARE, Europe) was preferred for stirring. An ultrasonication bath (P Selecta, Barcelona, Spain) was used for solving of samples.

Multivariate Analysis Add-in for Excel v1.3 software was used for all of the calculations in PLS-1 method (Brereton 2002).

Solutions

Solutions of MON (200 μg ml^{-1}), DES (200 μg ml^{-1}), and LEV (200 μg ml^{-1}) were prepared in methanol.

For preparing of pharmaceutical preparation solution, 10 film coated tablets containing a binary mixture of MON+LEV and MON+DES were accurately weighed and crushed in a mortar. Then, an equivalent amount of one tablet was dispersed in a 50-ml volumetric flask and diluted to volume with methanol. After 20 min of stirring, solutions were filtered from 0.45 μm Whatman No. 42 filtered paper. Following this, an appropriate amount of these filtrates was transferred into a 25-ml volumetric flask and diluted to volume with methanol, again. These solutions were used for further measurement in UV spectrophotometry.

Procedure

Optimization of PLS-1 method First, a training set containing ternary combinations of MON, DES, and LEV with different concentration values was established, and this is shown in Table 1. All of these solutions were taken into a UV spectrophotometry device, and absorbance values were measured in the range of 200–400 nm. Afterwards, ternary mixtures with their corresponding absorbances were placed into the chemometric software described in the "Apparatus" section. Their concentrations were noted for further evaluation of spectral data. Then, some experimental variables, such as type of chemometric method, wavelength ranges and numbers, number of principal component, data preprocessing techniques, and concentration values for each component especially for assays of ratio spectra derivative spectrophotometric method as a new preprocessing method, were optimized. During optimization studies, the marker of describing optimal conditions is the root mean square (RMS) values for each try, and this value has to be minimum.

Validation of optimized partial least squares method Working ranges are 2.4–20.0 μg ml^{-1} for MON, 2.0–12.0 μg ml^{-1} for DES, and 3.2–12.8 μg ml^{-1} for LEV. Limit of quantitation (LOQ) values are the minimum concentrations of these ranges.

Table 1 Training set used in PLS-1 method for MON, DES, and LEV in mixture.

No	MON (μg ml^{-1})	DES (μg ml^{-1})	LEV (μg ml^{-1})
1	2.4	7.2	3.2
2	20.0	2.0	3.2
3	20.0	12.0	6.4
4	11.2	4.8	16.0
5	7.2	12.0	9.6
6	7.2	7.2	6.4
7	16.0	4.8	6.4
8	20.0	4.8	12.8
9	16.0	12.0	3.2
10	2.4	2.0	12.8
11	11.2	9.6	3.2
12	16.0	2.0	9.6
13	16.0	7.2	12.8
14	7.2	9.6	12.8
15	2.4	9.6	6.4
16	7.2	4.8	3.2
17	11.2	2.0	6.4
18	2.4	4.8	9.6

The accuracy and precision were studied using three different solutions containing 7.2, 11.2 and 16.0 μg ml^{-1} of MON, 4.8, 7.2, and 9.6 μg ml^{-1} of DES, and 4.8, 6.4, and 9.6 μg ml^{-1} for LEV. Repeatability studies were achieved on different days.

Results and discussion

Optimization of PLSs method

Simultaneous determination of active ingredients in pharmaceutical preparations containing their mixture becomes more difficult with different analytical methods for possible interference effect of each ingredient. Hence, separation techniques such as chromatographic methods are widely used. Despite being powerful techniques, they have some disadvantages such as expensive tools and implements used and complicated instrumentation. Spectrophotometric measurements are easier and cheaper than chromatographic methods; besides, the UV spectrophotometric device is simpler than the chromatographic method. Notwithstanding, there are some difficulties in the case of overlapped spectra in mixtures, and simultaneous determination of each ingredient in the mixture is not possible with traditional spectrophotometric method such as measurement of absorbance values at maximum wavelength of each spectra. Besides derivative and ratio spectra-derived spectrophotometric methods, chemometric techniques are widely used for this type of analysis. In these techniques, using a wide range of wavelengths provides more knowledge for evaluation of spectral data. In this study, simultaneous determination of MON, DES, and LEV is not possible using traditional spectrophotometric methods in the range of 200–312 nm due to their overlapped spectrum even though determination of MON is possible in the range of 312–400 nm (Figure 2), and chemometric techniques were decided to be used in solving these overlapped spectra. For this purpose, PLS-1 method is preferred than the other techniques because of some of its important advantages like using processed data, usable full spectrum, and using principal components for prediction of concentration mainly.

After method choosing, suitable wavelength range and number of components were investigated in obtaining accurate and precise results for each component. RMS values were obtained with the minimum at 61 wavelengths with $\Delta\lambda$=1 nm within the range of 340–400 nm for MON, at 29 wavelengths with $\Delta\lambda$=1 nm within the range of 250–278 nm for DES, and at 23 wavelengths with $\Delta\lambda$=1 nm within the range of 218–240 nm for LEV. In these optimal conditions, RSM values are 0.07, 0.16, and 0.22 for MON, DES, and LEV, respectively. To select the number of principal components for modeling the system without overfitting the concentration data in PLS-1 algorithm, a cross-validation method, leaving out one sample at a time, was employed using training data

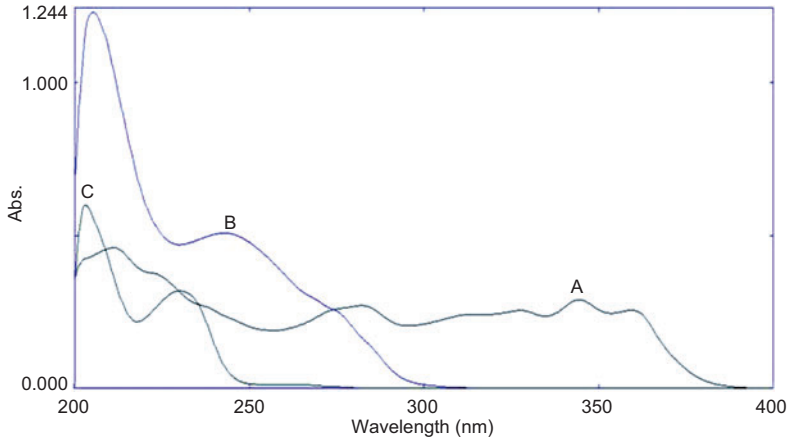

Figure 2 Zero-order absorption spectra of (A) 7.2 μg ml^{-1} solution of MON, (B) 12.0 μg ml^{-1} solution of DES, and (C) 9.6 μg ml^{-1} solution of LEV in methanol.

sets. Using this method, six principal components for MON and LEV and five principal components for DES were found to be optimal for the determination.

Data preprocessing is the important part of chemometric techniques. Generally, processed data are preferred than the nonprocessed data. In this study, standardized data for MON and LEV were used, while the nonpreprocessed data were chosen for DES. As an alternative to these traditional techniques, derivative and ratio spectra derivative spectrophotometric methods were evaluated new data preprocessing techniques. Hence, first of all, ternary mixtures of MON, DES, and LEV were divided into a sum of spectra containing different concentration values of two active ingredients. Then, the first-derivative spectrum of this divided spectra were taken. The sum of the spectrum of the solution of 4.8 µg ml^{-1} of DES and 7.2 µg ml^{-1} of MON for determination of LEV and the sum of the spectrum of the solution of 6.4 µg ml^{-1} of LEV and 7.2 µg ml^{-1} of MON for determination of DES were preferred as optimum divisor spectra, and with this combination, relative standard deviation values for validation set were obtained as minimum. The $\Delta\lambda$ found as optimum for the first derivative of their ratio spectra was 1 nm. While this process was used for DES and LEV, the first-derivative spectrophotometry was used for MON because there is no interaction from DES and LEV in the wavelength range of 340–400 nm. Standardized new processed values for LEV and MON that were in the same wavelength range described above for each component was evaluated for PLS-1 method. In contrast with this, raw data of the derivatized values of DES were used. All of these mathematical processes were done by Microsoft Excel 2007, and ratio spectra first-derivative spectrum of DES and LEV and first-derivative spectrum of MON are shown in Figure 3.

Validation of optimized PLSs method

The accuracy and precision were studied using three different solutions described in the "Experimental" section, and results are shown in Table 2. High recovery values and lower relative standard deviation indicate that these developed PLS-1 methods estimate the concentration values accurately and precisely for simultaneous determination of MON, DES, and LEV.

LOQ values are calculated described in the "Experimental" section and obtained as 2.4, 2.0, and 3.2 µg ml^{-1} for MON, DES, and LEV, respectively. The limit of detection (LOD)

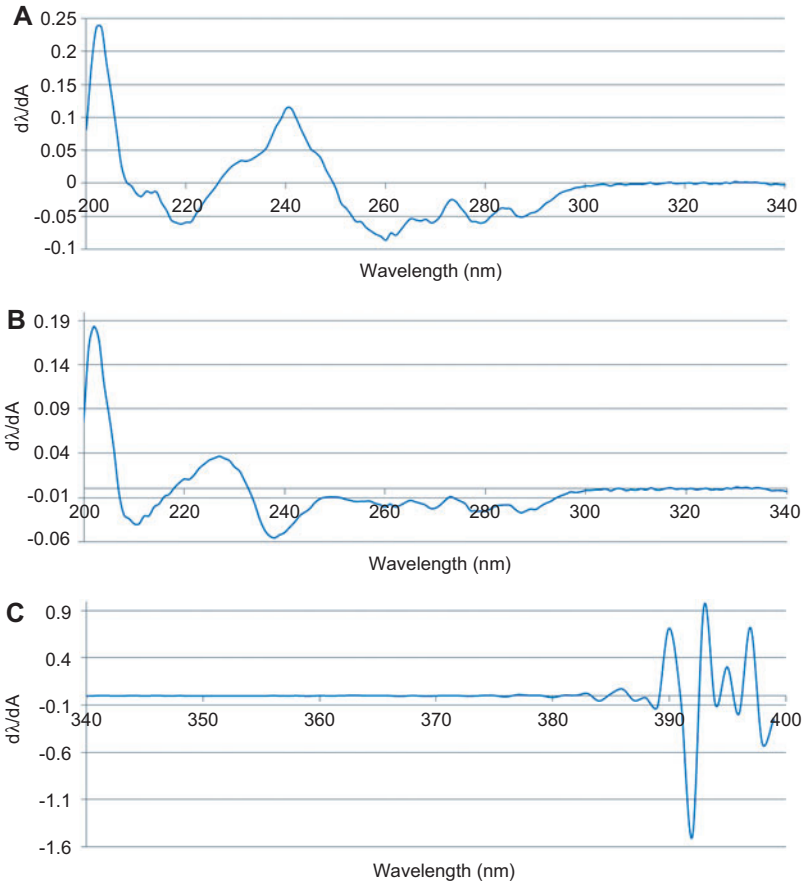

Figure 3 Ratio spectra first-derivative spectrum of (A) 12.0 µg ml^{-1} solution of DES, and (B) 9.6 µg ml^{-1} solution of LEV, and first-derivative spectrum of (C) 7.2 µg ml^{-1} solution of MON in methanol.

Table 2 Accuracy and precision data for MON, DES, and LEV.

Added (μg ml^{-1})	PLS-1[a]					
	Intraday			Interday		
	Found[b] (μg ml^{-1})	Precision (RSD[c])	Accuracy[d]	Found[b] (μg ml^{-1})	Precision (RSD[c])	Accuracy[d]
MON						
7.2	6.89±0.09	2.16	95.74±1.20	6.72±0.09	3.31	93.34±1.26
11.2	11.22±0.02	0.35	100.13±0.20	11.07±0.05	1.16	98.74±0.48
16.0	15.85±0.07	0.83	99.04±0.47	15.65±0.11	1.98	97.48±0.76
DES						
4.8	5.02±0.01	0.15	104.71±0.09	4.98±0.02	1.02	103.76±0.46
7.2	7.26±0.04	1.01	100.89±0.59	7.25±0.02	0.71	100.70±0.29
9.6	9.43±0.04	0.69	98.26±0.39	9.42±0.02	0.53	98.15±0.21
LEV						
6.4	6.43±0.03	0.91	100.43±0.53	6.38±0.04	1.67	99.69±0.08
9.6	9.64±0.01	0.06	100.42±0.04	9.52±0.05	1.17	99.19±0.47
12.8	13.06±0.11	1.42	102.05±0.84	12.87±0.05	0.92	102.05±0.84
Derivative spectrophotometry						
MON						
7.2	6.89±0.08	2.06	95.72±1.14	6.65±0.11	4.17	92.17±1.74
11.2	11.23±0.02	0.37	100.29±0.22	10.94±0.09	2.23	97.29±0.82
16.0	15.93±0.06	0.65	99.57±0.37	15.65±0.10	1.76	97.47±0.65
Ratio spectra derivative spectrophotometry						
DES						
4.8	5.02±0.01	0.28	104.71±0.17	5.02±0.04	1.73	104.58±0.74
7.2	7.25±0.01	0.24	100.64±0.14	7.25±0.02	0.74	100.62±0.30
9.6	9.49±0.04	0.74	98.84±0.42	9.52±0.02	0.53	99.15±0.25
LEV						
6.4	6.33±0.03	0.89	98.83±0.51	6.41±0.85	2.07	100.12±0.85
9.6	9.63±0.04	0.77	100.32±0.44	9.62±0.02	0.63	100.19±0.26
12.8	12.91±0.10	1.35	100.88±0.78	12.89±0.06	0.92	100.09±0.44

[a]In this study, standardized data for LEV and MON and nonpreprocessed data for DES were used. [b]Mean±standard error. [c]RSD%, relative standard deviation. [d]Accuracy%, Found/Added×100±standard error.

values were calculated in relation to figures of merit (FoM) (Kang et al. 2010) and obtained as 1.65, 0.64, and 0.70 μg ml^{-1} for MON, DES, and LEV, respectively.

Analysis of pharmaceutical preparation

First of all, for the investigation of any possible interference effect of excipients in the pharmaceutical preparation containing MON+LEV and MON+DES combinations, standard addition method was performed as a recovery test. The recoveries for this developed method obtained are summarized in Table 3. As shown in Table 3, these values are close to 100%. It is specified that there is no interference effect from the excipients in film-coated tablets. Then, sample solutions were prepared as described in the "Solutions" section. Satisfactory results were obtained for each compound and were found to be in agreement with the label claims (Table 4). The results obtained by the developed PLS-1 method were compared for all data-preprocessing technique, and no significant difference was observed for the amount of drugs using the Student t-test and the Fisher F-test at the p=0.05 level in commercial formulations. Using the ratio spectra derivative spectrophotometric

Table 3 Recovery results obtained from standard addition method for DES and LEV in binary combinations with MON in pharmaceutical formulations.

	MON		DES		MON		LEV	
	PLS-1	^{1}D[a]	PLS-1	^{1}DD[b]	PLS-1	^{1}D[a]	PLS-1	^{1}DD[b]
Mean±SE[c]	99.01±0.41	98.98±0.52	97.11±1.16	94.33±1.14	95.35±1.06	96.65±1.01	97.90±1.06	107.59±1.29
RSD[d]	0.82	1.06	2.92	2.96	2.71	2.57	2.66	2.93

[a]^{1}D, first-derivative spectrophotometry. [b]^{1}DD, ratio spectra first-derivative spectrophotometry. [c]Mean±standard error. [d]RSD%, relative standard deviation.

Table 4 Assay results of commercial preparations (DESMONT® (5 mg desloratadine and 10 mg montelukast sodium/film-coated tablet, batch no: 01090) and LEVMONT® (5 mg levocetirizine hydrochloride and 10 mg montelukast sodium/film-coated tablet, batch no: 01060).

	DESMONT®			
	MON		DES	
	PLS-1	^{1}D[a]	PLS-1	^{1}DD[b]
Mean±SE[c]	10.24±0.04	10.32±0.09	4.98±0.04	4.96±0.04
RSD[d]	0.91	1.97	1.94	1.71
Accuracy[e]	102.37±0.42	103.18±0.91	99.60±0.86	99.24±0.76
	t-test			
	0.82		0.31	
	F-test			
	4.73		1.29	
	LEVMONT®			
	MON		LEV	
	PLS-1	^{1}D[a]	PLS-1	^{1}DD[b]
Mean±SE[c]	10.06±0.06	10.26±0.12	4.86±0.06	4.84±0.05
RSD[d]	1.43	2.69	1.94	2.44
Accuracy[e]	100.58±0.64	102.62±1.43	97.29±1.26	96.74±1.05
	t-test			
	1.47		0.34	
	F-test			
	3.71		1.07	

[a]^{1}D, first-derivative spectrophotometry. [b]^{1}DD, ratio spectra first-derivative spectrophotometry. [c]Mean±standard error. [d]RSD%, relative standard deviation. [e]Accuracy, added/found×100±standard error. Theoretical value for t at p: 0.05 level=2.31; theoretical value for F=6.3.

method for data processing, relative standard deviation values were fewer than when using other data preprocessing techniques for DES and LEV. These results indicate that derivative and ratio spectra derivative spectrophotometric methods will be used as new data preprocessing methods in chemometric techniques from now on.

References

Aronson, J. K. Meyler's Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions; Elsevier: Amsterdam, **2006**.

Brereton, R. G. Chemometrics, Data Analysis for The Laboratory and Chemical Plant; John Wiley & Sons: New York, NY, **2002**.

Dhandayuthan, S.; Satheesh, B.; Sree, G. K. K. Simultaneous RP-HPLC quantification of desloratadine and montelukast sodium in a tablet formulation. *Res. J. Chem. Environ.* **2011**, *15*, 815–818.

Kang, G.; Lee, K.; Park, H.; Lee, J.; Jung, Y.; Kim, K. Quantitative analysis of mixed hydrofluoric and nitric acids using Raman spectroscopy with partial least squares regression. *Talanta* **2010**, *81*, 1413–1417.

Nayak, A. S.; Schenkel, E. Desloratadine reduces nasal congestion in patients with intermittent allergic rhinitis. *Allergy* **2001**, *56*, 1077–1080.

Philip, G.; Malmstrom, K.; Hampel, F. C.; Weinstein, S. F.; LaForce, C. F.; Ratner, P. H. Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. *Clin. Exp. Allergy* **2002**, *32*, 1020–1028.

Rote, A. R.; Niphade, S. V. Determination of montelukast sodium and levocetirizine dihydrochloride in combined pharmaceutical dosage form by RP-HPLC. *Lat. Am. J. Pharm.* **2010**, *29*, 1020–1023.

Rote, A. R.; Niphade, S. V. Determination of montelukast sodium and levocetirizine dihydrochloride in combined tablet dosage form by HPTLC and first-derivative spectrophotometry. *J. Liq. Chromatogr. R. T.* **2011**, *34*, 155–167.

Topuz, B.; Ogmen, G. G. Montelukast as an adjuvant to mainstay therapies in patients with seasonal allergic rhinitis. *Clin. Exp. Allergy* **2003**, *33*, 823–826.

Received July 26, 2011; accepted January 7, 2012;
previously published online February 26, 2012