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Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods

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Abstract

In this study, high performance liquid chromatography (HPLC) and second derivative spectrophotometry have been used and described for the simultaneous determination of montelukast and loratadine in pharmaceutical formulations. HPLC separation was achieved with a Symmetry C18 column and sodium phosphate buffer (pH adjusted to 3.7): acetonitrile (20:80, v/v) as eluent, at a flow rate of 1.0 ml/min. UV detection was performed at 225 nm. The LC method is simple, rapid, selective and stability indicating for the determination of montelukast. 5-Methyl 2-nitrophenol was used as internal standard for the purpose of quantification of both the drugs in HPLC. In the second-order derivative spectrophotometry, for the determination of loratadine the zero-crossing technique was applied at 276.1 nm, but for montelukast peak amplitude at 359.7 nm (tangent method) was used. Both methods were fully validated and a comparison was made for assay determination of selected drugs in formulations. The results confirm that the methods are highly suitable for its intended purpose.

Keywords: Montelukast; Loratadine; HPLC; Derivative spectrophotometry; Assay

1. Introduction

Montelukast sodium, a specific cysteinyl leukotriene receptor antagonist, belongs to a styrylquinolines series with the chemical name 2-[1-[1(*R*)-[3-[2(*E*)-(7-chloroquinolin-2-yl)vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl)phenyl]propylsulfanylmethyl]cyclopropyl] acetic acid sodium salt (Fig. 1). It is developed as a therapeutic agent for the treatment of bronchial asthma by Merck & Co. [1].

Loratadine is a non-sedating H_1-antihistamine with a chemical name 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine carboxylic acid ethyl ester (Fig. 1). It is a potent and orally active, that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis by Schering–Plough Company.

Merck & Co. and Schering–Plough are jointly pursuing the development and marketing of a

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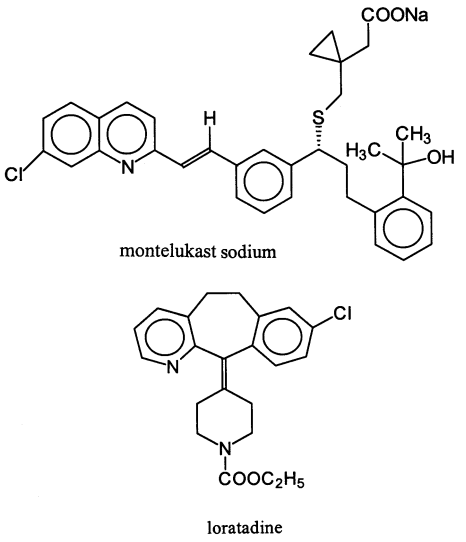

Fig. 1. Structures of montelukast sodium and loratadine.

once-daily fixed-combination tablet containing montelukast sodium and loratadine for the treatment of allergic and asthma [2].

Only a few chromatographic methods have been reported for the determination of montelukast in the open literature. Furthermore, the determination of montelukast in human plasma using fluorescent detection [3], residual acetate analysis in bulk drug [4], its *S*-enantiomer in human plasma by stereoselective high performance liquid chromatography (HPLC) using column-switching [5] and its determination in human plasma by column-switching HPLC method [6] have been described.

Many chromatographic methods have been published for the determination of loratadine in biological fluids and commercial formulations [7–19].

The direct UV–Vis spectrophotometric methods give a severe problem in that the spectral bands of the co-formulated drugs overlap, which makes the simultaneous determination of these drugs impossible. The derivative technique in UV spectrophotometry has been utilized successfully to overcome the problem of interference due to spectral overlapping. Besides, this technique offers a powerful enhancement of sensitivity and rapid determination without the requirement of extraction or separation and providing accurate, precise and reproducible results [20,21]. This ability has been conveniently applied for the determination of active ingredients in content uniformity tests, in dissolution tests and in other similar in-process tests of pharmaceutical formulations.

No spectrophotometric method or HPLC method has been reported in the literature for the determination of montelukast, nor for the simultaneous determination of montelukast and loratadine in their commercial formulations.

It would therefore be beneficial to provide accurate, precise and reliable methods for determining montelukast and for simultaneous determination of montelukast and loratadine.

The present work describes two analytical procedures for the quantitation of montelukast in its single dosage form or in co-formulation with loratadine using reversed phase HPLC and second derivative spectrophotometry.

2. Experimental

2.1. Chemicals

Samples of montelukast and loratadine were received from Vorin Laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile was obtained from Merck & Co., USA. Tablets of Singulair (equivalent to 5 mg of montelukast) were procured from Merck & Co. Tablets of Lorfast (equivalent to 10 mg of loratadine) of Le Santé (a division of Cadilla Pharmaceuticals Ltd., India) were purchased from the market. Analytical grade sodium di-hydrogen phosphate was obtained from Pro Analysi, E Merck (India) Ltd. Ortho phosphoric acid was obtained from Qualigens, India.

High purity water was prepared by using Waters Milli-Q plus purification system.

2.2. Instrumentation

2.2.1. High performance liquid chromatography

The LC system consisted of a Waters 510 pump, a Rheodyne injector equipped with a 10 μl sample loop, and a Photodiode array detector (Waters 996). The output signal was monitored and

integrated using Millenium 2010 Chromatography Manager software (WATERS).

2.2.2. Derivative UV spectrophotometry

Spectrophotometric analysis was performed on Perkin–Elmer lambda 35—a computer controlled double beam UV–Visible spectrophotometer using 10 mm quartz cells with a slit width of 1 nm and scan speed of 60 nm/min.

2.3. Solutions

2.3.1. Liquid chromatography

2.3.1.1. Mobile phase preparation. The mobile phase consisted of a mixture of aqueous 0.025 M sodium dihydrogen phosphate (buffer pH adjusted to 3.7 using dilute orthophosphoric acid) and acetonitrile in the ratio 20:80 (v/v). The mobile phase was filtered through a 0.45 μm nylon membrane filter and degassed prior to use.

2.3.1.2. Sample preparation. Sample solutions of montelukast and loratadine were prepared in mobile phase. The working concentration for the determination of assay of both drugs was 400 μg/ml. The concentration of the internal standard was maintained at 330 μg/ml in each combined solution of montelukast and loratadine that were used for quantitative studies. All the volumetric flasks containing montelukast sodium were wrapped with black paper and stored in the dark.

2.3.2. Derivative spectrophotometry

2.3.2.1. Preparation of standard solutions of montelukast and loratadine. Stock solutions of montelukast and loratadine were prepared by accurate weighing of 25 mg in each case and dissolving in a mixture of acetonitrile and water in the ratio 80:20 (v/v) and then made up to 25 ml in volumetric flasks. For simultaneous quantitative studies of both drugs, a series of standard solutions containing both the drugs were prepared by appropriate dilution of mixture of stock solutions. All the volumetric flasks were wrapped with black paper.

2.3.2.2. Preparation of montelukast and loratadine samples from Singulair and Lorfast tablets. Twenty tablets of Sigulair (each tablet containing 5 mg of montelukast) and Lorfast (equivalent to 10 mg of loratadine present in tablet) in each case were weighed and ground to powder separately. Accurately weighed powder samples equivalent to 10 mg of montelukast and 10 mg loratadine were transferred to a 50 ml volumetric flask. About 40 ml of solvent consisting a mixture of acetonitrile and water in the ratio 80:20 (v/v) was added to the flask and placed in an ultrasonic bath at room temperature for 15 min. The solution was cooled and made up to volume and then centrifuged using Biofuse-13 centrifuger. The supernatant was taken into a clean dry flask, tightly capped and wrapped with black paper and placed in the dark. Different sample solutions of required concentrations were prepared using supernatant.

2.4. Conditions

2.4.1. Chromatographic conditions

A Symmetry C_{18}, 250 × 4.6 mm, 5 μm, analytical column (Waters make) was used for the separation. The mobile phase was delivered through the column at a flow rate of 1.0 ml/min. Column was operated at ambient temperature (∼ 22 °C). The sample injection volume was 10 μl. The photodiode array detector was set at a wavelength of 225 nm.

2.4.2. Measurement of derivative spectra

Second derivative UV spectra for the solutions of montelukast and loratadine were recorded in a 10 mm cell over the range 250–375 nm using acetonitrile–water mixture (in the ratio 80:20, v/v) in the reference cell. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

3. Results and discussion

3.1. HPLC

3.1.1. Method development

For HPLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between montelukast, loratadine and internal standard. The mobile phase consisting of 0.025 M sodium dihydrogen phosphate buffer (pH 3.7) and acetonitrile in the ratio 20:80 (v/v) was found to be an appropriate mobile phase allowing adequate separation of all the compounds using a Symmetry C18 250 mm column at a flow rate of 1.0 ml/min. A typical chromatogram of separation of all the compounds is shown in Fig. 2.

As montelukast and loratadine exhibit significant absorbance at wavelength 225 nm, it was selected as detection wavelength for the simultaneous determination of montelukast and loratadine in pharmaceutical dosage forms. In the present method the selectivity was found to be more than 1.85 with a resolution more than 6.6 for all the three compounds. System-suitability results of the method are presented in Table 1.

3.1.1.1. Purity determination of montelukast. High–low chromatography technique [22] was employed for purity determination of a real sample of montelukast. In this technique a sample containing high concentration of montelukast was injected without considering about going above the linear range of main component (API). The sensitivity towards low-level impurities thus was increased by more than five times and the response of each impurity was recorded. Weight percentage of each impurity present in the sample was

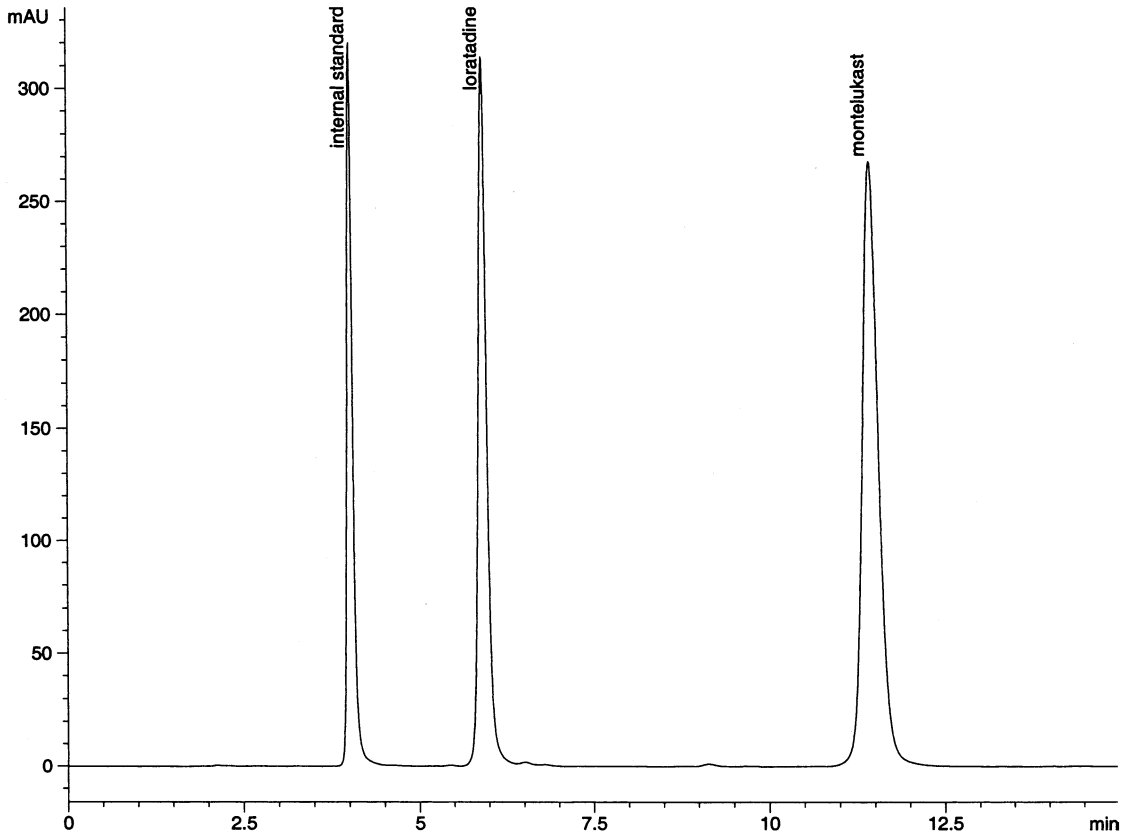

Fig. 2. HPLC chromatogram of montelukast, loratadine and internal standard.

Table 1
System-suitability report of HPLC method

Compound	Capacity factor[a]	Selectivity[a]	Tailing factor[a]	Resolution[a]	Theoretical plates[a]
Internal standard	1.13	–	1.36	–	5987
Loratadine	2.05	1.85	1.29	6.63	6884
Montelukast	5.45	2.66	1.18	15.81	9897

[a] Number of samples analyzed is three.

calculated by comparing its response to response of dilute montelukast (0.5%), prepared from concentrated sample (5 mg/ml). A chromatogram of montelukast using high–low chromatography and its corresponding spectrum index plot using PDA detection is shown in Fig. 3. The impurities namely UK1, UK2, and UK3 eluted at retention times 4.9, 5.2 and 32.3 min, respectively, were not characterized (Fig. 3).

3.1.2. Method validation

The developed method was validated for simultaneous assay determination of montelukast and loratadine using following parameters. 5-Methyl 2-nitrophenol was used as internal standard for the purpose of quantification of co-formulated drugs.

3.1.2.1. Specificity. Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. Complete separation and resolution of impurities, loratadine and internal standard from montelukast peak with good peak shapes and without any apparent shoulders confirms specificity of the method (Figs. 2 and 3).

3.1.2.1.1. Preparation of degraded samples. Accelerated degradation studies were also performed to demonstrate the validity of the method.

For acid degradation, montelukast sample was refluxed with 0.1 N HCl at 60 ± 1 °C for 12 h (flask was wrapped with aluminum foil to protect from light) and then neutralized by adjusting pH to 7.0 with NaOH. The solution was further diluted to required concentration with the mobile phase. For basic degradation, montelukast sample was refluxed with 0.1 N NaOH at 60 ± 1 °C for 12 h (flask was wrapped with aluminum foil to protect from light) and then neutralized by adjusting pH to 7.0 with HCl. The solution was further diluted to required concentrations. For photo and thermal degradations, two separate solutions of montelukast (400 μg/ml in mobile phase) were prepared. One solution was exposed to ultraviolet light (254 nm) for 24 h, and the other was kept at 50 °C temperature for 12 h (flask was wrapped with aluminum foil). The sample refluxed with 0.1 N HCl showed little degradation and the degraded products were well separated from the analyte peak. But with 0.1 N NaOH refluxing, montelukast didnot give any significant degradation. The sample exposed to UV light was mostly converted to its *cis*-isomer (II). On the other hand, the heated sample did not give any major degradation products. All the degraded samples were subjected to photo-diode array analysis for peak purity [23]. The plot reports showed that montelukast had no detectable impurity peaks embedded in them and are free of co-eluting degradation compounds. It is clear that the method can be used for determining the stability of montelukast in bulk and pharmaceutical formulations. Selectivity of loratadine with respect to its degradation has not been studied.

3.1.2.2. Linearity. Linearity was checked by preparing standard solutions of both montelukast and loratadine at five different concentration levels in the same volumetric flasks using their respective stock solutions. The calibration curves for montelukast and loratadine were drawn in the concentration range of 100–600 and 116–580 μg/ml, respectively. The regression equation, for calibration curve of montelukast is $y = 2.59x - 0.006$ and for loratadine is $y = 1.83x + 0.0008$. The average slope values of montelukast and loratadine were 2.593 ± 0.09 and 1.829 ± 0.044, respectively.

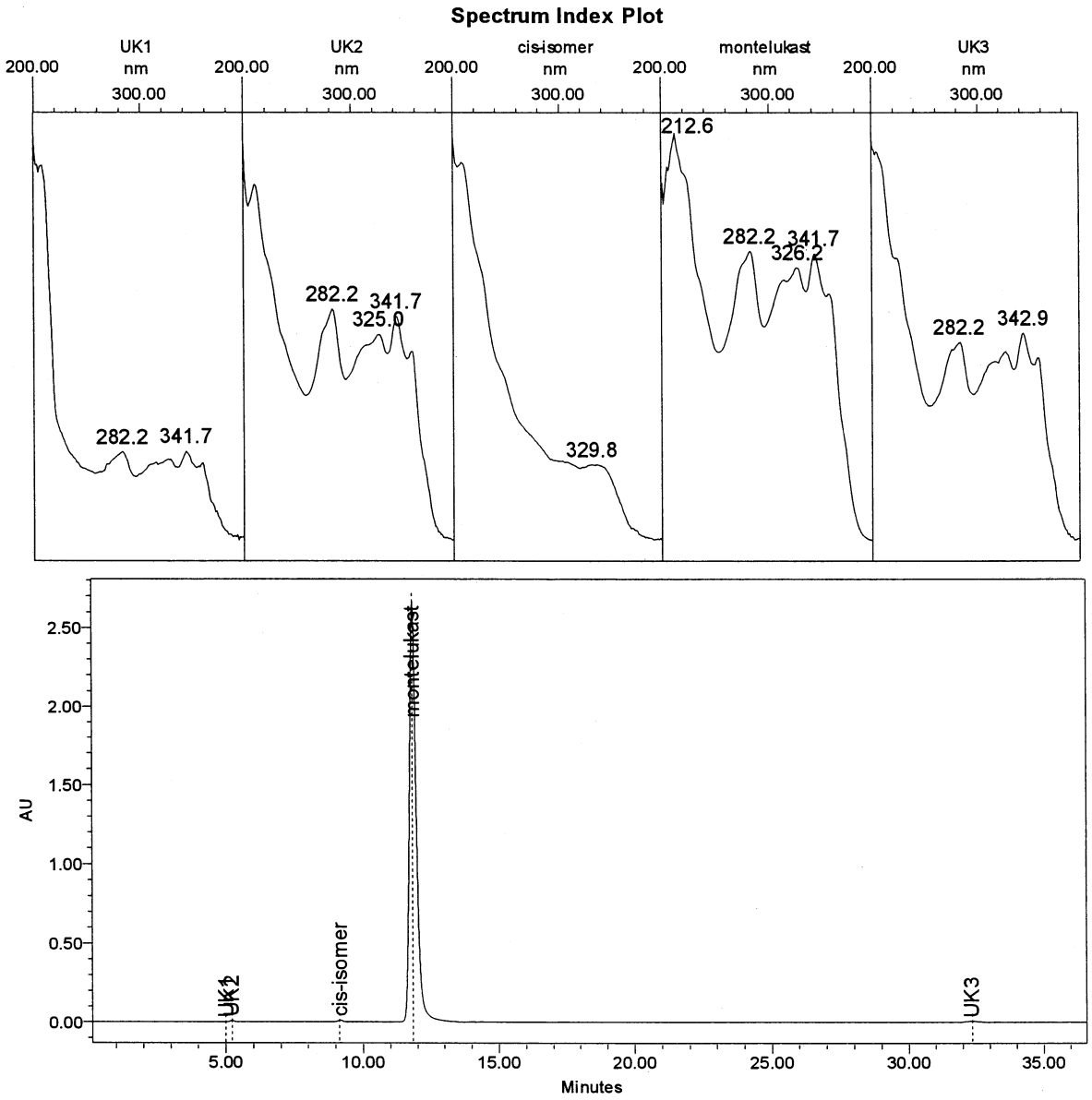

Fig. 3. High–low chromatogram of montelukast with spectrum index plot for all compounds using PDA detection.

3.1.2.3. Precision and accuracy. Intra-day precision and accuracy of the method were evaluated for synthetic mixtures of montelukast and loratadine at three different independent concentrations i.e. 150, 300 and 450 μg/ml ($n = 3$) by determining their assay. The R.S.D. values ranged from 0.37 to 1.41 and 0.34 to 1.17% for montelukast and loratadine, respectively (Table 2). Inter-day precision and accuracy of the method was tested for 3 days at the same concentration levels. Solutions for calibration curves were prepared fresh every day. The R.S.D. values ranged from 0.44 to 0.68 and 0.45 to 0.66% for montelukast and loratadine, respectively (Table 2). Percentage recoveries ran-

Table 2
Precision and accuracy in the assay of montelukast and loratadine by HPLC

Day	Mean concentration of montelukast (mg/ml) ($n = 3$)		Percentage of recovery of montelukast	RSD for Montelukast determination (%)	Mean concentration of loratadine (mg/ml) ($n = 3$)		Percentsge recovery of loratadine	RSD for loratadine determination (%)
	Taken	recovered			Taken	recovered		
1	0.148, 0.302, 0.461	0.147, 0.302, 0.461	99.32, 100.11, 100.10	1.18, 1.01, 0.66	0.152, 0.308, 0.442	0.150, 0.309, 0.440	98.46, 100.32, 99.47	0.77, 1.17, 0.35
2	0.148, 0.302, 0.461	0.148, 0.304, 0.464	99.77, 100.55, 100.72	1.41, 0.82, 0.45	0.152, 0.308, 0.442	0.151, 0.310, 0.442	99.34, 100.75, 99.90	0.66, 0.98, 0.34
3	0.148, 0.302, 0.461	0.149, 0.305, 0.465	100.90, 100.88, 100.86	1.02, 0.50, 0.37	0.152, 0.308, 0.442	0.152, 0.312, 0.444	100.22, 101.19, 100.37	1.03, 0.81, 0.35
Inter-day	0.148, 0.302, 0.461	0.148, 0.304, 0.463	100.0, 100.55, 100.43	0.68, 0.50, 0.44	0.152, 0.308, 0.442	0.151, 0.310, 0.442	99.34, 100.65, 100.0	0.66, 0.49, 0.45

ging from 99.3 to 100.9 and 98.4 to 101.2 were obtained for montelukast and loratadine, respectively (Table 2).

3.1.3. Assay determination of montelukast and loratadine from formulations

3.1.3.1. Tablets of montelukast: extraction. Twenty weighed chewable tablets of Sigulair (equivalent to 5 mg of montelukast) were ground to a fine powder. An amount of powder equivalent to 50 mg of montelukast was accurately weighed and extracted with mobile phase in a 25 ml flask using ultrasonicator. The solution was centrifuged using Biofuse-13 centrifuger. The supernatant was taken into a clean dry flask and wrapped with black paper.

3.1.3.2. Tablets of loratadine: extraction. Twenty tablets of Lorfast were weighed and ground well thoroughly. The powder equivalent to 50 mg of loratadine was extracted with mobile phase in a 25-ml flask and centrifuged using Biofuse-13 centrifuger. The supernatant was taken into a clean dry flask and tightly capped.

3.1.3.3. Simultaneous quantification of montelukast and loratadine. 0.25, 0.5, 0.75 and 1.0 ml volumes of the supernatants of both the tablets were transferred into 5.0 ml flasks; 1.0 ml volumes of the internal standard solution were added and made up with mobile phase. Then, 10 μl volume of each sample was injected into the column.

The percentage recoveries of montelukast and loratadine from tablets ranged from 99.04 to 100.47% with R.S.D. values less than 1.46 and 98.98 to 101.02% with an R.S.D. value less than 1.54%, respectively (Table 3).

3.1.4. Stability

The stability of montelukast in solution containing mobile phase and the internal standard was determined for the samples stored at normal light conditions (tightly capped flasks kept on bench top) and under reduced light conditions (tightly capped flasks were wrapped with aluminum foil). The samples were checked for 3 days of storage and the data were compared with freshly prepared

samples. Solutions kept under reduced light conditions were found stable and the R.S.D. values of assay were well below 2.0% against freshly prepared samples. The montelukast solutions kept under normal light conditions are getting degraded to give *cis*-isomer impurity with time.

3.2. Derivative spectrophotometric method

3.2.1. Simultaneous assay determination of both montelukast and loratadine

The zero-order absorption spectra of montelukast and loratadine solutions in 80% acetonitrile are shown in Fig. 4. The spectra display overlapping in the region of 200–300 nm. This makes the determination of loratadine in the presence of montelukast by conventional UV spectrophotometry difficult, but the determination of montelukast from 310 to 375 nm might be possible without the interference from loratadine. The derivative spectrophotometry technique was, however, chosen for the determination of both the drugs since it could remove broadband contributions from excipients and might also overcome the interference from peak overlapping. Derivative spectra of different orders were studied for both montelukast and loratadine individually and simultaneously. The experiments showed that the second derivative spectra of montelukast and loratadine were simple, and gave results with suitable precision at $\Delta\lambda$ value 4 nm. In this second derivative spectra the signals at 276.1 nm (zero-crossing point of montelukast) are proportional to the loratadine concentration and the signals at 359.7 nm (loratadine reads zero) are proportional to the montelukast concentration (Fig. 4). Tangent method was used for the measurement of peak amplitude at 359.7 nm (of montelukast).

3.2.2. Calibration graph

To obtain calibration curves for both montelukast and loratadine, the second derivative spectra of standard drug solutions in combination were recorded at five varied concentrations of montelukast (4.4–22.1 μg/ml) and loratadine (6.4–32.1 μg/ml) (Fig. 5). The spectrum was measured three times for each concentration. The equation for calibration curve of montelukast is $y = 3.36x +$

Table 3
Simultaneous determination of montelukast and loratadine by using HPLC and second derivative methods

Drug	Using HPLC method			Using second derivative method		
	Taken (mg)	Recovery (mean ±S.D.) ($n = 3$)	Percentage recovery	Taken (μg)	Recovery (μg) ($n = 3$) (mean ± t(S.D./$\sqrt{n}$))[a]	Percentage recovery
Montelukast	0.105, 0.209, 0.314, 0.418	0.104 ±0.0015, 0.210 ±0.0027, 0.315 ± 0.002, 0.419 ±0.0041	99.05, 100.47, 100.32, 100.24	5.12, 7.62, 9.87	5.04 ±0.05, 7.74 ±0.09, 10.03 ± 0.07	98.43, 101.57, 101.62
Loratadine	0.098, 0.197, 0.295, 0.394	0.099 ±0.0015, 0.196 ±0.0027, 0.292 ± 0.0015, 392 ±0.0047	100.02, 99.50, 99.00, 99.50	10.11, 15.23, 20.84	9.95 ±0.07, 15.38 ±0.20, 20.94 ±0.22	98.41, 100.98, 100.48

[a] S.D., standard deviation; t, 4.30 (at 95% confidence limits and $n-1$ degrees of freedom).

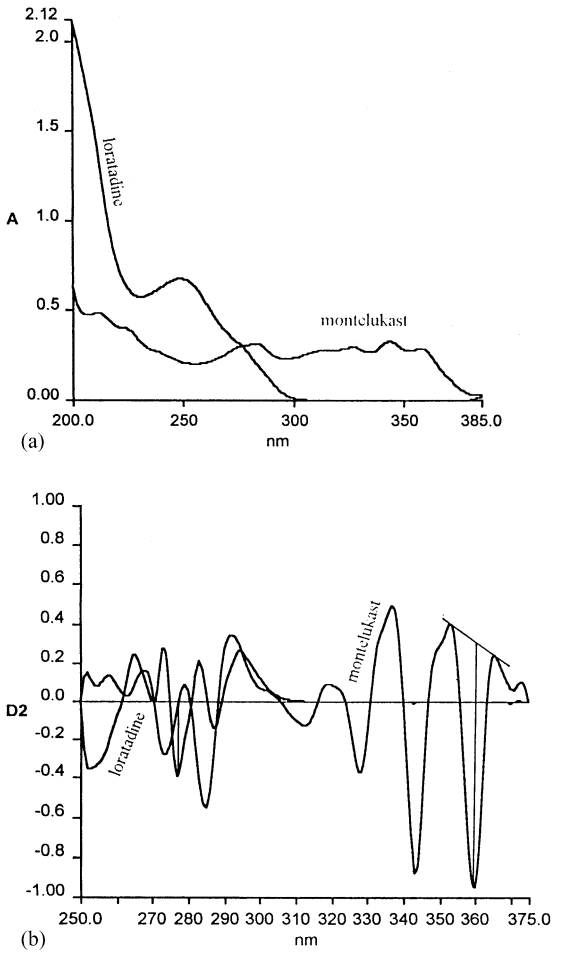

Fig. 4. Zero-order (a) and second-order (b) spectra of montelukast and loratadine in water:acetonitrile (20:80).

1.31 and for loratadine is $y = 0.653x + 0.39$. The correlation coefficients of calibration plots were equal to 0.998, indicating good linearity in both cases.

3.2.3. Accuracy

Accuracy of the second derivative method for simultaneous determination of montelukast and loratadine was checked at independently prepared concentration levels at 5, 7.5 and 10 μg and 10, 15 and 20 μg, respectively, ($n = 3$). The mean recovery data for each level (at 95% confidence limits) and its percentage recoveries are presented in Table 3.

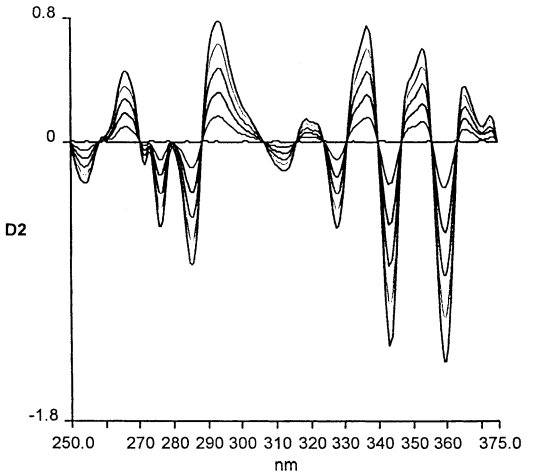

Fig. 5. Second-order derivative spectra of binary mixture of montelukast (4.4, 8.8, 13.3, 17.7 and 22.1 μg/ml) and loratadine (6.4, 12.8, 19.3, 25.7 and 32.1 μg/ml) in water:acetonitrile (20:80).

3.3. Application to dosage forms

The performance of the derivative spectrophotometric method was statistically compared with that of the HPLC method by Student's t-test and the F-test at 95% confidence level. The calculated t and F-values (0.61 and 2.7, and 0.1 and 3.3 for montelukast and loratadine, respectively) did not exceed the theoretical values ($t = 2.31$, $F = 9.6$; $n = 5$), indicating that there was no significant difference between derivative spectrophotometric method and HPLC methods with regard to accuracy and precision. Further, to check the validity of the proposed methods, the standard addition method was applied by adding different amounts of montelukast with concentration range 50–150 μg/ml (for HPLC) and 5–15 μg/ml (for D2); and loratadine with concentration range 35–105 μg/ml (for HPLC) and 7–21 μg/ml (for D2) to the previously analyzed tablets. The mean recoveries of the added drugs were found to be 99.82 ± 0.62 (for HPLC), 100.56 ± 0.83 (for D2) and 100.22 ± 0.74 (for HPLC), 100.93 ± 0.67 (for D2) for montelukast and loratadine, respectively. The results of analysis of the commercial tablets and the recovery study (standard addition method) suggested that there is no interference from any excipients present in the tablets.

4. Conclusion

HPLC and second derivative spectrophotometric methods were developed and validated for the simultaneous determination of montelukast and loratadine in pharmaceutical dosage forms. The methods were found to be simple, precise and rapid. The assay results obtained by these two methods are in fair agreement. The HPLC method may offer advantages over the derivative spectrophotometric method for selective determination of the montelukast in the presence of their degradation products or in a variety of matrices. The derivative spectrophotometric method serves as an alternative method for the determination of both montelukast and loratadine in commercial samples. These methods can be used for the routine determination of montelukast and loratadine in release testing.

References

[1] D.F. Schoors, M.D. Smet, T. Reiss, D. Margolskee, H. Cheng, P. Larson, R. Amin, G. Somers, Br. J. Clin. Pharmacol. 40 (1995) 277.
[2] http://DailyDrugNews.com (Daily Essentials), May 24 (2000).
[3] R.D. Amin, H. Cheng, J.D. Rogers, J. Pharm. Biomed. Anal. 13 (1995) 155–158.
[4] M.M. See, R. Thompson, N. Grinberg, H.J. Perpall, G. Bicker, P. Tway, J. Liq. Chromatogr. 18 (1) (1995) 137.
[5] L. Liu, H. Chang, J.J. Zhao, J.D. Rogers, J. Pharm. Biomed. Anal. 15 (5) (1997) 631–638.
[6] H. Ochiai, N. Uchiyama, T. Takano, K. Hara, T. Kamei, J. Chromatogr. B 713 (1998) 409–414.
[7] R. Johnson, J. Christensen, C.C. Lin, J. Chromatogr. B 657 (1) (1994) 125.
[8] R. Johanson, K. Lin, J. Christensen, J. Diancin, J. Phram. Res. 11 (10) (1994) Abst APQ1209.
[9] D. Zhong, H. Blume, Pharmazie 49 (10) (1994) 736–739.
[10] J. Martens, F.P. Meyer, U. Troger, T. Franke, Br. J. Clin. Pharmacol. 39 (5) (1995) 560.
[11] Z. Chen, R. Shi, W. Gee, E.T. Lin, J. Phram. Res. 13 (9) (1996) Abst APQ 1153.
[12] N.M. Najib, M.S. Salem, G.K. Pillia, J.A. Jilani, M.M. Hasan, F.M. Zghol, E. Ganem, I. Jalal, J. Phram. Res. 13 (9) (1996) Abst APQ 1110.
[13] P.K. Kunicki, J. Chrmatogr. B 755 (1–2) (2001) 331–335.
[14] F.C.W. Sutherland, A.D. de Jager, D. Badenhorst, T. Scanes, H.K.L. Hundt, K.J. Swart, A.F. Hundt, J. Chromatogr. A 914 (1–2) (2001) 37–43.
[15] M. Vasudevan, S. Ravisankar, A. Sathiyanarayanan, R.S. Chandan, Indian Drugs 38 (5) (2001) 276–278.
[16] K. Gangaram, V. Bhanu Raman, Indian Drugs 37 (12) (2000) 574–576.
[17] F.J. Rupérez, H. Fernández, C. Barbas, J. Pharm. Biomed. Anal. 29 (2002) 35–41.
[18] N.A. El-Ragehy, A.M. Badawey, S.Z. Elkateeb, J. Pharm. Biomed. Anal. 28 (2002) 1041–1053.
[19] T. Radhakrishna, J. Satyanarayana, A. Satyanarayana, Indian Drugs 39 (6) (2002) 342–347.
[20] A.F. Fell, UV Spectrom. Group Bull. 8 (1980) 5.
[21] G. Talsky, Derivative Spectrophotometry, Low and High Order, VCH, Weinheim, 1994.
[22] H. Anthony, T. Scoutt, K.E. Dean, Talanta 40 (1993) 491–494.
[23] F.B. Ibrahim, J. Liquid Chromatogr. 18 (13) (1995) 2621–2633.