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A Validated Comparitave LC and Ratio First Derivative Spectrophotometric Method for the Simultaneous Determination of Levocetirizine dihydrochloride and Montelukast sodium in Bulk and Pharmaceutical dosage forms

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ABSTRACT

A new simple, rapid, precise reverse phase-high performance liquid chromatographic (RP-HPLC) and ratio spectra first derivative spectroscopy (¹DD) methods has been developed for the simultaneous determination of Levocetirizine dihydrochloride (Levo) and Montelukast sodium (Mont) in bulk active pharmaceutical ingredient (API) as well as in tablet dosage form. In RP-HPLC method, separation was performed using phenomex-luna 5 μ C8 (2) (100Å, 250 X 4.6 mm) column by using acetonitrile: 0.5% triethylamine in water (90:10 v/v) pH adjusted to 5.5 $\pm$ 0.1 with orthophosphoric acid. The flow rate was 0.8 ml/min with UV detection monitored at 231 nm. The retention time was 3.8 and 5.2 min for Levo and Mont respectively. In ratio spectra first derivative method, linearity range was found to be 2-32 μ g/mL and 3-30 μ g/mL for Levo and Mont respectively. From the first derivative (¹DD) suitable wavelength was selected and amplitudes were measured at 240 nm and 281 nm for the assay of Levo and Mont by considering concentration of 18 μ g/mL of Mont and 24 μ g/mL of Levo as a suitable divisor, respectively. The validation of method was carried out according to ICH guidelines.

Keywords: HPLC, Ratio spectra first derivative, Levocetirizine dihydrochloride, Montelukast sodium.

INTRODUCTION

Levo, 2-[2-[4-[(R)-(4-chlorophenyl)-phenyl methyl] piperazinyl-1-yl] ethoxy] acetic acid (Figure.1a), R-enantiomer of racemic cetirizine, is a selective, potent, H1-antihistamine antagonist indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria. It has a rapid onset, achieving maximum plasma concentration (t_{max}) in 0.9 h, with peak serum levels (C_{max}) of approximately 270ng/mL (Maryadele, *et al.*, 2006, Tripathi, 2008, and Hair, *et al.*, 2006). The drug undergoes minimal metabolism, which increases the bioavailability with 8 h elimination half-life. Levo is generally well tolerated in adults, adolescents and children with allergic conditions (Passalacqua *et al.*, 2005).

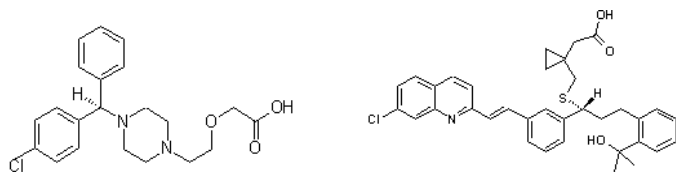


Fig. 1: Structure Of Levocetirizine Dihydrochloride And Montelukast Sodium.

Mont, 2-[1-[(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl -sulfanylmethyl] cyclopropyl] acetic acid sodium salt (Figure.1b) is a fast acting and potent cysteinyl leukotriene receptor antagonist which is being used in the treatment of asthma (Schoors, *et al.*, 1995). It can be administered orally once daily thereby increasing compliance over other common asthma treatments, has no known adverse effects or drug interactions, has demonstrated efficacy against allergen or exercise-induced bronchoconstriction (EIB) and is the only leukotriene modifier approved by the US FDA for use by children from 2 to 12 years of age (Hansen-Flaschen, 1998). A rapid onset of action is seen after the administration of Mont with improvement on the first day of treatment (Knorr, *et al.*, 1998), and these positive effects may be additive to those of inhaled corticosteroids (Wenzel, *et al.*, 1998). Mont has been demonstrated to provide superior protection compared to the long acting inhaled β -agonist, salmeterol (Bronskey, *et al.*, 2000). In the study by Leff 1998 (Leff *et al.*, 1998) neither tolerance to the medication nor rebound worsening of lung function after discontinuation of the Mont were seen. While inhaled β -agonists are still considered the first-line therapy for treatment of asthma, Mont may be given due consideration for use as first line therapy in patients with mild persistent asthma, for additional control in those who remain symptomatic during treatment with inhaled corticosteroids, for patients those are steroidphobic or for those who have difficulties with compliance (Blake, *et al.*, 1999). This combination is indicated for relief of symptoms of allergic rhinitis (seasonal and perennial). In the study conducted by Ciebiada 2008 (Krawiec, *et al.*, 1999 and Ciebiada, *et al.*, 2008) on quality of life in patients with persistent allergic rhinitis, the benefits of this combination were evident in most domains measured by rhinoconjunctivitis quality of life questionnaire (RQLQ), specifically in allergic rhinitis symptoms. Also in the study by Vipan 2010 (Viapan, *et al.*, 2010), efficacy of Levo and Mont as treatment for allergic rhinitis, proving this combination was very effective in improving primary outcome of daytime nasal symptom scores (PDTS) in patients of allergic rhinitis. Most interesting aspect is these two drugs are formulated in a single dosage form, in a manner to minimize observed interaction between the two drugs. There have been several reports on this combination providing synergistic effect for the treatment or prevention of inflammation, asthma or allergic disorder. Literature survey reveals few sensitive and selective methods based on HPLC and UV derivative spectrophotometry for estimation of title drugs individually and in combination with other drugs (Shamkant, *et al.*, 2009, Radhakrishna, *et al.*, 2003, Ambadas, *et al.*, 2010, and Choudhari, *et al.*, 2010). Bioanalytical methods have been reported for the determination of Levo and

Mont individually in different biological matrices like plasma by column switching HPLC method with fluorescence detection (Hisao, *et al.*, 1998 and Ibrahim, 2004), voltametric method (Alsarra, *et al.*, 2005), and LC/MS (Robert, *et al.*, 2007 and Morita, *et al.*, 2008). In the present study, we described a comparative validated RP-HPLC and ratio first derivative spectrophotometric method for the simultaneous determination of Levo and Mont.

MATERIALS AND METHODS

Experimental

Apparatus and Softwares

Shimadzu UV 1601 double beam spectrophotometer connected to an IBM compatible computer loaded with Shimadzu UVProbe 2.10 software (Shimadzu Corporation, Kyoto, Japan) was used for all the spectrophotometric measurements. The spectral bandwidth was 1 nm and the wavelength scanning speed was 2800 nm min⁻¹.

The adsorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200-350 nm. The HPLC system was Shimadzu class LC-10A (Japan), including pump LC-10AT, SPD-10A vp UV-VIS detector and the Spinchrom CFR software-single channel was used for acquisition, evaluation and storage of chromatographic data. Different equipments like analytical weighing balance (Shimadzu AUX 220), sonicator (SONICA 2200 MH), pH meter, vacuum filter pump (model XI 5522050 of Millipore) were used.

Chemicals and reagents

The drug samples Levo and Mont were supplied by Biocon Ltd, Bengaluru, Karnataka, India, as a gift samples. It was certified to have a purity of 99.07% and 99.85% of Levo and Mont respectively. Methanol and acetonitrile were of HPLC grade (Spectro Chem. Ind. Ltd.) and all other chemicals used were of analytical grade. Water of ultrapure grade of 18 M Ω resistance was obtained in-house from Millipore Milli-Q plus water purification system (Millipore, Milford, MA, USA) were used in the analysis.

Procedure

Standard stock and working solutions

Levo and Mont standard stock solution: 1 mg/mL in methanol and acetonitrile solvents prepared separately for spectroscopy and HPLC respectively. Working stock solutions of 0.40 and 0.60 mg/ml prepared separately by transferring 2.0 and 3.0 ml of Levo and Mont from the standard stock into two different 50 ml volumetric flask's each and diluted to volume with methanol and acetonitrile for spectroscopy and HPLC use respectively.

Extraction of Levo and Mont from pharmaceutical tablets:

Twenty tablets [Montek-LC, Montair LC (Levo 5 mg, Mont 10 mg)] were accurately weighed and average weight of tablets was determined and pulverized to fine powder. Fine powder equivalent to two tablets were transferred separately into 100 mL volumetric flasks. Added 5 ml of methanol to each flask and then about 50 ml of suitable solvents (methanol for spectroscopy use &

acetonitrile for HPLC use as indicated above) were added and sonicated for 20 min, after the ensuring complete solubilization, then the solutions are filtered through Whatmann filter paper No: 44. The residues were washed twice with 20 ml portions of solvent and final volume was made with respective solvents for spectroscopy and HPLC use.

Ratio first derivative spectra method

The ratio spectra of different Levo standards at increasing concentration in methanol obtained by dividing each with the stored spectrum of the standard solution of 18 µg/ml Mont as divisor spectra. The first derivative (¹DD) of this spectrum traced with interval of $\Delta\lambda=8$ nm are illustrated in Figure 2. As seen in Figure 2 there exist one minimum (240 nm) and one maximum (225 nm) and found that both were suitable for determination of Levo in Levo and Mont mixture. The wavelength 240 nm selected for the determination of this compound in the assay of synthetic mixtures, tablets, due to its lower R.S.D values and more suitable mean recovery compared with other wavelength. For the determination of Mont, the ratio spectra of different Mont standards at increasing concentrations in methanol obtained by dividing each with stored spectrum of the standard solution of 24 µg/mL of Levo as divisor spectra. The first derivative (¹DD) of this spectrum traced with interval of $\Delta\lambda=8$ nm are illustrated in Figure 3. As seen in Figure 3 there exist one minimum (298 nm) and one maximum (281 nm) and in this also both were suitable for the determination of Mont in Levo and Mont mixture. The peak at wavelength 281nm was selected because of its lower R.S.D and more suitable mean recoveries. All the optimized parameters are summarized in the Table 1.

Table. 1: Ratio ¹DD Spectra optimized method parameters.

Method parameters	Optimized Values
Solvent	Methanol
Scanning range (nm)	200 nm to 400 nm
Slit width	2 nm
Scan speed	Fast (2800 nm min ⁻¹)
Smoothing factor ($\Delta\lambda$)	8 nm
Scaling factor	100
Analytical wavelength for determination of Levo	240 nm
Analytical wavelength for determination of Mont	281 nm

METHOD VALIDATION

The proposed method was validated as per ICH 2005 guidelines (ICH, 2005)

Linearity

To evaluate linearity of the methods, different concentration of the analytes in the range of 2-32 µg/ml for Levo and 3-30 µg/ml for Mont was analyzed (Table 2) and the linearity between the concentration and peak-area in HPLC, absorbance in spectroscopy were examined for each analyte. The results obtained shows that the current methods are linear for the analytes in the range specified above with a correlation coefficient of better than 0.999.

LOD and LOQ

In RP-HPLC a signal-to-noise ratio 3:1 and 10:1 is considered for calculating LOD and LOQ respectively. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. In ratio first derivative spectroscopy, calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows $LOD=3.3*SD/slope$ of calibration curve, $LOQ= 10*SD/slope$ of calibration curve. The values of LOD and LOQ are given in Table 2.

Table. 2: Linear regression data for the standard curves (n=6)&LOD and LOQ parameters.

Parameter	RP-HPLC		RATIO SPECTRA(¹ DD)	
	LEVO	MONT	LEVO	MONT
Wavelength (nm)	231	231	240	281
Linearity and range (µg mL ⁻¹)	2-32	3-30	2-32	3-30
Correlation coefficient (r^2)	0.9997	0.9994	0.9995	1.000
Intercept (m)	0.451	0.6323	0.208	461.44
Slope (c)	0.0063	0.1316	0.0394	79.032
LOD (µg/mL ⁻¹)	0.00028	0.0032	0.2979182	0.3177621
LOQ (µg/mL ⁻¹)	0.00086	0.0094	1.8263959	2.4459207

Precision

The precision of the both (HPLC, ¹DD) proposed methods was determined by studying the intra-day and inter-day precision (expressed in, % RSD) which was performed on three concentrations between the linearity range in five replicates. % RSD for both intraday and inter-day precision was found to be less than 2.0 % in both the methods proving that the proposed methods are precise (Table 3).

Accuracy

The recovery study was done by following standard addition technique in both cases. The extracted formulation samples were spiked with 50, 100 and 150 % of the standard Levo and Mont and the mixtures was analyzed by the proposed methods. The experiment was conducted in five replicates. This was done to check the recovery of the drug at different levels in the formulations. Results have shown that the mean recovery of the assay is within $100 \pm 2.0\%$ for each ingredient, and % RSD is lower than 2.0 % (Table 4).

Robustness

Robustness of the HPLC method measures its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability. By taking one concentration (8 µg/ml) from linearity range the solution was subjected to deliberate variation in the method i.e., slight change of mobile phase ratio, detection wavelength and change in flow rate was done and the effect of deliberate variations was expressed in % RSD (Table 5). The Robustness of the spectroscopy method was determined by using methanol from three different manufacturers for the preparation of stock solutions of formulation and standard drugs. The average value of % RSD of the responses was $\leq 2.0 \%$.

Table. 3: Intra and Inter day precision of LEVO and MONT.

Analytes	HPLC						RATIO SPECTRA ^(1DD)					
	Conc (µg/ml)		LEVO area	MONT area	%RSD*		Conc (µg/ml)		LEVO abs	MONT abs	%RSD*	
	Levo	Mont			Levo	Mont	Levo	Mont			Levo	Mont
Intra-day	4	8	1.215	4.954	1.773	1.456	2	3	0.353	1114.9	0.639	0.421
	10	20	3.764	12.692	1.764	1.145	12	21	2.353	5340.9	0.885	0.099
	15	30	5.968	19.639	0.690	0.590	32	30	5.573	11090	0.166	0.413
Inter- day	4	8	1.412	4.919	1.319	1.403	2	3	0.359	1115.6	0.696	0.765
	10	20	3.743	12.690	1.408	1.398	12	21	2.314	4835.1	0.041	1.492
	15	30	5.931	19.469	1.586	1.483	32	30	5.573	9635.2	0.133	1.313

*Mean of six replicate readings (n=6)

Table. 4: Percentage recovery.

Analytes	HPLC			RATIO SPECTRA(¹ DD)		
	Excess drug added to analyte (%)	conc	% Recovery ± %RSD*	Excess drug added to analyte (%)	conc	% Recovery ± %RSD*
LEVO	50%	12	101.422 ± 0.050	50%	7.5	102.90 ± 0.6
	100%	16	100.63 ± 0.104	100%	10	99.79 ± 0.12
	150%	20	101.422 ± 0.050	150%	12.5	102.63 ± 0.13
MONT	50%	9	98.17 ± 0.15	50%	15	101.68 ± 0.02
	100%	12	101.30 ± 0.19	100%	20	98.752± 0.12
	150%	15	98.17 ± 0.15	100%	25	101.72± 0.10

*Mean of three replicate readings

Table. 5: Robustness, on evaluation of the HPLC method.

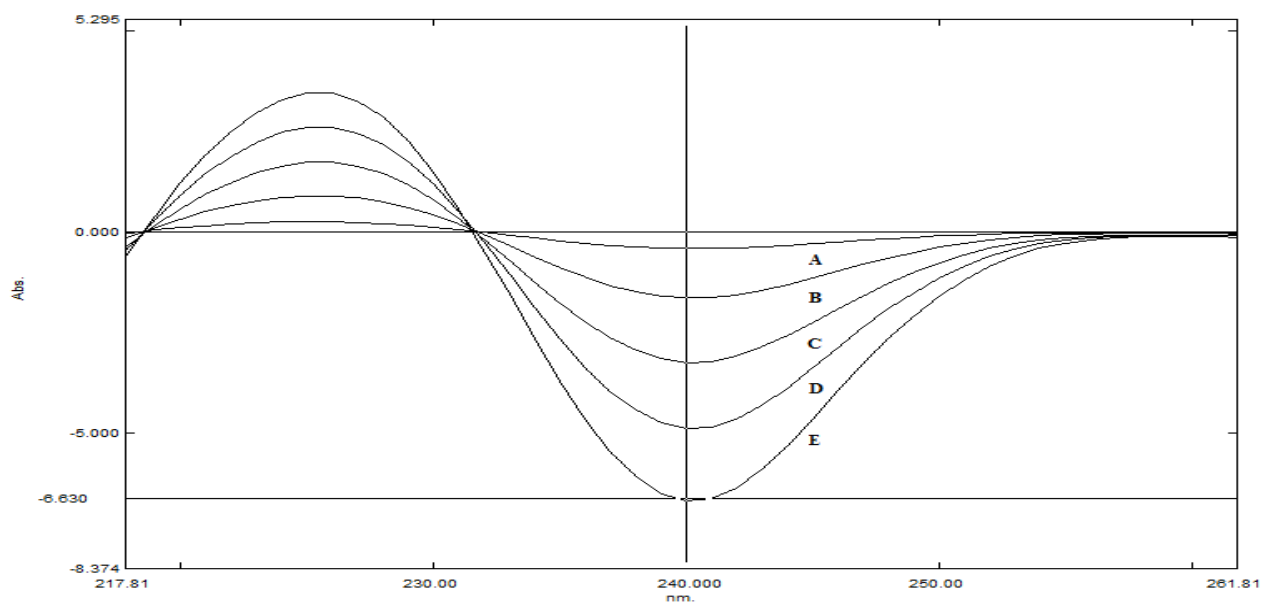
Parameters*	Change in flow rate (±) 0.1ml/min			Acetonitrile (±)5% in mobile phase ratio			Change in wavelength (±)1nm		
	0.7	0.8	0.9	85:15	90:10	95:5	230	231	232
LEVO	Rt (min)	3.682	3.853	3.941	3.583	3.854	3.746	3.811	3.853
	Area (mV)	1.030	1.215	1.281	0.997	1.215	1.112	1.143	1.215
	% RSD	1.2258	1.2040	1.520	1.444	1.2040	1.121	1.3414	1.2040
MONT	Rt (min)	5.172	5.223	5.32	5.271	5.223	5.229	5.111	5.223
	Area (mV)	4.756	4.854	4.964	4.756	4.854	4.964	4.522	4.854
	% RSD	1.2091	1.473	1.690	1.218	1.473	1.345	1.3425	1.473

*Mean of three replicate readings

Table. 6: Results of analysis of commercial formulation

Tablets	HPLC			RATIO SPECTRA ^(1DD)		
	Label claim (mg)	Drug content (%) ± SD	%RSD*	Label claim (mg)	Drug Content (%) ± SD	% RSD*
Montek-LC	5	99.18 ± 0.89	0.68	5	101.34 ± 0.64	0.42
	10	100.26 ± 1.73	0.26	10	99.68 ± 1.14	0.38
Montair LC	5	99.83 ± 1.048	0.57	5	100.64 ± 0.34	0.52
	10	98.28 ± 1.253	0.74	10	99.48 ± 0.86	0.35

*Mean of three replicate determinations

**Fig. 2:** Ratio spectra first derivative of LEVO a) 2, b) 8, c) 16, d) 24, e) 32, µg/ml solution of LEVO when 18 µg/ml of MONT used as divisor ($\Delta\lambda = 8\text{nm}$) ; scaling factor, 100.

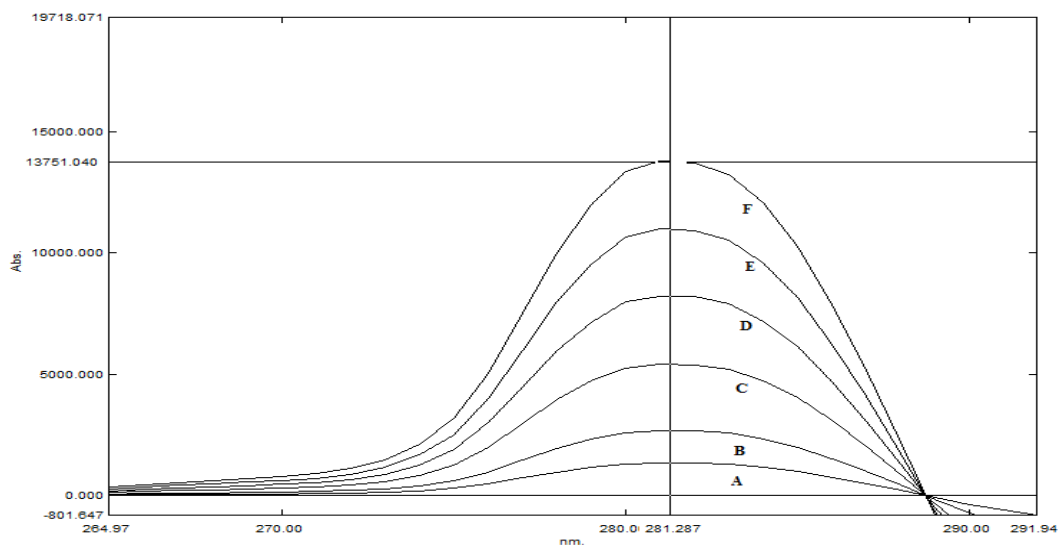


Fig. 3: Ratio spectra first derivatives of MONT (a) 2 (b) 4 (c) 8 (d) 12 (e) 16 (f) 20 (g) 24 µg/ml solution of MONT when 24 µg/ml of LEVO used as divisor ($\Delta\lambda = 8\text{nm}$); scaling factor, 100.

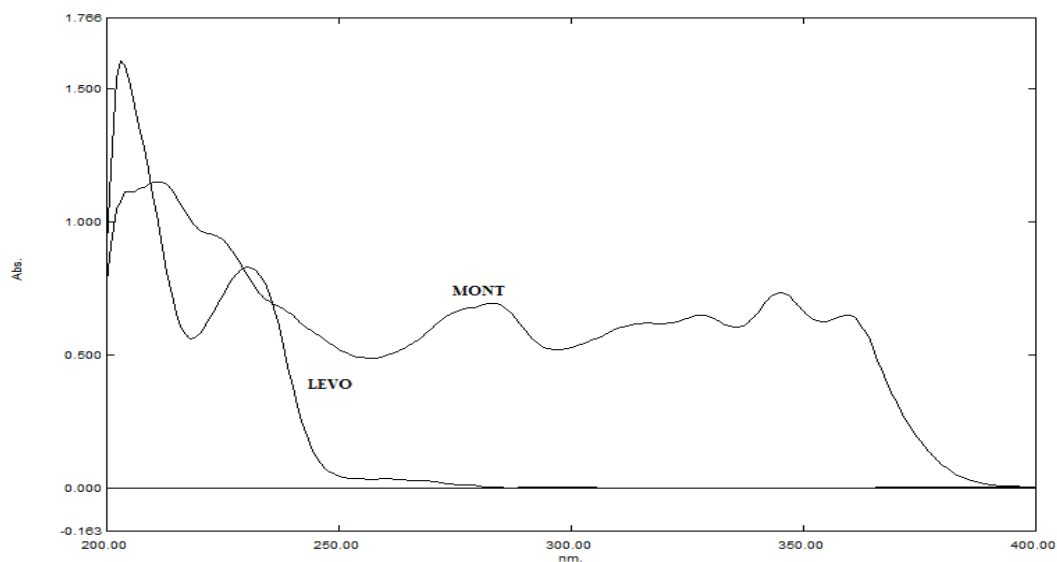


Fig 4. Ratio spectra first derivatives of formulations (a) LEVO 24 µg/ml as divisor (b) MONT 18 µg/ml as divisor.

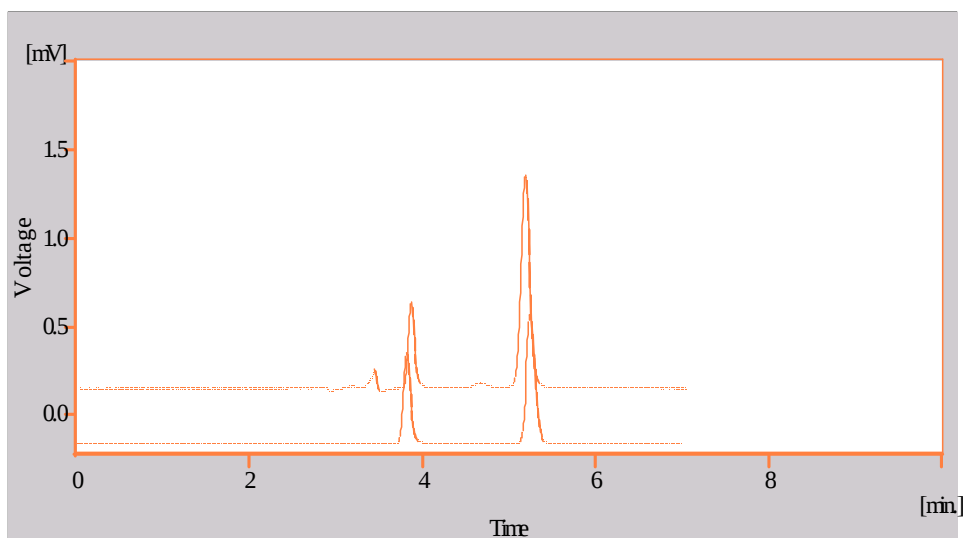


Fig. 5: Typical 3D HPLC Chromatogram of STD (1st) and Tested Samples (2nd) of the entitled drugs

The recovery study was done by following standard addition technique in both cases. The extracted formulation samples were spiked with 50, 100 and 150 % of the standard Levo and Mont and the mixtures was analyzed by the proposed methods. The experiment was conducted in five replicates. This was done to check the recovery of the drug at different levels in the formulations. Results have shown that the mean recovery of the assay is within $100 \pm 2.0\%$ for each ingredient, and % RSD is lower than 2.0 % (Table 4).

Robustness

Robustness of the HPLC method measures its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability. By taking one concentration (8 µg/ml) from linearity range the solution was subjected to deliberate variation in the method i.e., slight change of mobile phase ratio, detection wavelength and change in flow rate was done and the effect of deliberate variations was expressed in % RSD (Table 5). The Robustness of the spectroscopy method was determined by using methanol from three different manufacturers for the preparation of stock solutions of formulation and standard drugs. The average value of % RSD of the responses was $\leq 2.0\%$.

Specificity

Specificity of the methods was demonstrated by good separation of the two analytes from each other (Figure 5). Furthermore, excipient of the tablet formulation did not interfere with the active ingredients of the drug product, in both spectroscopy and Chromatography method.

Analysis of the marketed formulation:

Applicability of the proposed methods was tested by analyzing the commercially available tablet formulation Montek-LC (Mfg: Sun pharmaceuticals, Sikkim, Batch No. BSJ1939) and Montair LC tablets labeled to contain 5 mg of Levo and 10 mg of Mont for both the methods. In HPLC 20 µl of the tablet extracts (8, 12 µg/ml of Levo and Mont respectively) was injected. In Spectroscopy the extracted solutions was further diluted to the desired concentration ranges and the followed method is applied. The possibility of excipient interference in both the analysis was studied.

RESULTS AND DISCUSSION

Optimization of Method

RP-HPLC

The main objective while developing this method was to separate two drugs. Initially acetonitrile: methanol (50:50 v/v) was tried for each drug individually. Peak splitting was observed for Levo, then 0.25% formic acid in methanol: acetonitrile was tried in the ratio of 80:20 (v/v) which resulted in splitting of Mont peak. Then formic acid was replaced by 0.5% triethylamine in water and pH range 4.0 to 6.0 adjusted with orthophosphoric acid in different ratios was tried.

Finally, a mobile phase comprising of acetonitrile: 0.5% v/v triethylamine in water in the ratio (90:10) pH adjusted to 5.5 with orthophosphoric acid with isocratic elution showed typical good peak nature and peak symmetry at 231 nm for both the drugs. The flow rate of the mobile phase was finalized to 0.8 ml/min. Under optimized conditions the tailing factor for both peaks was less than 1.2 with satisfactory resolution.

Ratio first derivative spectra

Ratio derivative method permits the determination of components in mixtures at wavelengths corresponding to a maximum or minimum. The values at these points permit better sensitivity and accuracy. The main instrumental parameters that affect the shape of the derivative ratio spectra are the concentration of divisor spectra, smoothing ($\Delta\lambda$) and scaling factor. The effects of these parameters were studied and fast scanning speed, smoothing factor ($\Delta\lambda=8$), scaling factor (100) was selected and other optimized method parameters are summarized in Table.1. Divisor concentration is main instrumental parameter, the standard spectra of 18 µg/ml of Levo and 24 µg/ml of Mont was considered as divisor for the determination of Levo and Mont in mixtures respectively.

Calibration curves

The linear regression data for the calibration plots of HPLC ($n=6$) as shown in (Table 2, Fig-3) illustrates a good linear relationship over a concentration range of 1.5-6.0 µg/spot for Levo and 0.4-1.6 µg/spot for Mont with respect to the peak area. The regression data of 1DD spectra also showed a good linearity as shown in Table.2.

Application of the developed methods for analysis of commercial formulations

Applicability of the proposed methods tested analyzing the commercially available tablet formulations Montek-LC and Montair LC labeled to contain 5 mg of Levo and 10 mg of Mont. There was no interference from the excipients present in the tablet in both the methods. The results of the both proposed methods were very close to each other with satisfactory results in a good agreement with the label claims suggesting suitability of these methods. The results are discussed in the Table 6.

CONCLUSION

The developed HPLC and the ratio spectrophotometric methods were in good agreement with its proving results than other methods described in various papers for the simultaneous determination of Levocetizine dihydrochloride and Montelukast sodium in bulk and formulations without the interference of excipients proving the specificity of the methods. The ratio spectra derivative method is rapid, simple and sensitive owing to the advantages of the selectivity of suitable divisor concentration. HPLC method gave a good resolution between Levo and Mont within a short analysis time of (≤ 6 mins). The developed HPLC method is thus considered to be more specific than various other

methods published due to its short run time, specificity and robust values. Both the developed methods may be recommended for routine analysis and in any quality control set-up providing all the parameters are followed accurately for its intended use.

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REFERENCES

- Maryadele TN, Patricia EH, Cherie BK, and Kristin JT. The Merk Index. An encyclopedia of chemicals, drugs and biologicals. Merck reasearch labs. 14th ed. Merck & Co., Inc, White House station, New Jersey (2006) 2022, 6258
- Tripathi KD. Essential of medical pharmacology. 6th ed. Jaypee brothers medical publishers, New Delhi, India (2008) 159, 222-23
- Hair PL, Scott LJ. Levocetirizine: a review of its use in the management of allergic rhinitis and skin allergies. *Drugs*. 2006 66: 973.
- Passalacqua G., Canonica GW. A review of the evidence from comparative studies of levocetirizine and desloratadine for the symptoms of allergic rhinitis. *Clinical Therapeutics*. 2005 27: 979.
- Schoors DF., Smet MDR, Margolskee T, Cheng DHP, Larson R, Amin and Somers GB. *Journal of Clinical Pharmacology*. 1995 40: 277-280.
- Hansen-Flaschen J., Schotland HNE. New Treatments for Exercise-Induced Asthma. *Journal of Medicine*. 1998 339: 192-193.
- Knorr BM., Bernstein J, Nguyen J, Seidenberg H, Reiss B, Becker AT. Montelukast for chronic asthma in 6 to 14 year old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. *The Journal of the American Medical Association*. 1998 279: 1181- 6.
- Wenzel SE., Antileukotriene Drugs in the Management of Asthma. *The Journal of the American Medical Association*. 1998 280: 2068.
- Bronsky J., Grossman JP, Kemp AF, Ghannam PT, DeLucca GJ, Gormley and Pearlman DS. Oral Montelukast Compared with Inhaled Salmeterol To Prevent Exercise-Induced Bronchoconstriction a Randomized, Double-Blind Trial. *Annals of Internal Medicine*. 2000 132: 97.
- Leff JA., Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, Dockhorn, Kundu RS, Zhang J, Seidenberg BC, Reiss TF. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise - induced bronchoconstriction. *Journal of Medicine*. 1998 339: 147.
- Blake KV., Montelukast: data from clinical trials in the management of asthma. *The Annals of Pharmacotherapy*. 1999 33: 1299-1314.
- Krawiec ME., Wenzel SE. Leukotrienes: The Role of Leukotrienes in the Pathophysiology of Asthma and Allergic Rhinitis. *Current Opinion in Pediatrics*. 1999 11: 540.
- Ciebiada M., Gorska CM, Kmiecik T, DuBuske L, Gorski P. Quality of Life in Patients With Persistent Allergic Rhinitis Treated With Montelukast Alone or in Combination With Levocetirizine or Desloratadine. *Journal of Investigational Allergology and Clinical Immunology*. 2008 18:343-349.
- Vipan G., Matreja PS. Efficacy of Montelukast and Levocetirizine as Treatment for Allergic Rhinitis. *Journal of Allergy and Therapy*. 2010 1:103.
- Shamkant SP., Shinde A, Bavaskar S, Mandrupkar SN, Pandurang ND, Bhanudas SK. Development and statistical validation of spectrophotometry method for estimation of Montelukast in bulk and tablet dosage form. *Journal of Pharmacy Research*. 2009 2: 714-716.
- Radhakrishna T., Narasaraju A, Ramakrishna M, Satyanarayana A. Simultaneous determination of Mont and loratidine by HPLC and derivative spectroscopy. *Journal of Pharmaceutical Biomedical Analysis*. 2003 31: 359-368.
- Ambadas RR., Vaishali SN. Determination of Montelukast sodium and Levocetirizine dihydrochloride in combined Pharmaceutical dosage form by RP-HPLC. *Latin American Journal of Pharmacy*. 2010 29:1020-3.
- Choudhari V., Kale A, Abnawe S, Kuchekar B, Gawli V, Patil N. Simultaneous determination of Montelukast sodium and Levocetirizine dihydrochloride in pharmaceutical preparations by ratio derivative spectroscopy. *International Journal of PharmTech Research*. 2010 1:4-9.
- Hisao O., Naotaka U, Terukazu T, Ken-ichi H, Toshio K. A column switching HPLC method with fluorescence detection of MONT in human plasma. *Journal of Chromatography B*. 1998 713: 409-414.
- Ibrahim AA., Development of a stability-indicating HPLC method for the determination of montelukast in tablets and human plasma and its applications to pharmacokinetic and stability studies. *Saudi Pharmaceutical Journal*. 2004 12: 4.
- Alsarra I., Al-Omar M, Gadkariem EA, Belal F. Voltametric determination of MONT in plasma by voltametric method. *Il Farmaco*. 2005 60: 563-567.
- Robert P., Pauline L, Wayne M, Mullett, Elizabeth K. Quantification of MONT in sheep plasma by LC/MS. *Journal of Chromatography B*. 2007 858: 282-286.
- Morita MR., Berton D, Boldin R, Barros FAP, Meurer EC, Amarante AR, Campos DR, Abib JE, Pedrazolli JJ. Determination of LEVO in plasma by LC\MS. *Journal of Chromatography B*. 2008 862: 132-139.
- ICH, Validation of Analytical Procedures: Text and Methodology (Q2A (R1)): 2005 International Conference on Harmonization. IFPMA, Geneva.