ACTA CHROMATOGRAPHICA, NO. 19, 2007

SIMULTANEOUS DETERMINATION OF LORATADINE AND PRESERVATIVES IN SYRUPS BY THIN-LAYER CHROMATOGRAPHY

G. Popović, *M. Čakar*, and *D. Agbaba*[*]

Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P.O. Box 146, 11000 Belgrade, Serbia

SUMMARY

Simple, rapid, and precise densitometric TLC methods have been established for simultaneous determination of loratadine and preservatives in loratadine–sodium benzoate and loratadine–methylparaben–propylparaben mixtures. Chromatography was performed on aluminium plates precoated with silica gel 60 F_{254}. The mobile phases were *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*) for loratadine–sodium benzoate mixtures and ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*) for loratadine–methylparaben–propylparaben mixtures. Chromatographic zones were scanned in reflectance–absorbance mode at 240 nm for the former mixture and 275 nm for the latter. Relationships between peak areas and amounts of the compounds were evaluated by linear regression analysis in the concentration ranges 0.3–0.7 μg per band for loratadine, methylparaben, and sodium benzoate and 0.03–0.07 μg per band for propylparaben. Mean recovery for all the compounds varied from 98.3 to 102.5 %. Detection and quantification limits ranged from 0.004 to 0.03 and from 0.01 to 0.1 μg per band, respectively. The proposed method was used for simultaneous determination of loratadine and the preservatives in commercial medicinal syrups.

INTRODUCTION

Loratadine (ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidine)-1-piperidine carboxylate) is one of a group of second-generation antihistamines which resemble each other more in their pharmacological action than their chemical structures. These compounds are selective and moderately strong antagonists of histamine H_1 receptors. Because of the slow dissociation of the antihistaminic-H_1 receptor comp-

lex, and the formation of active metabolites with similar action on this class of histamine receptor, most of these drugs have prolonged antihistaminic action [1]. A very important feature of this generation of antihistamines is that when used at therapeutic concentrations they have a poorly expressed sedating effect, because only small amounts of the drugs penetrate the blood–brain barrier [2]. Loratadine is commercially available in the form of tablets and syrups, the latter always containing different preservatives.

Loratadine has been analysed in pharmaceutical formulations alone [3–10], in the presence of its degradation product [11], in mixtures with related impurities [12–14], and with other drugs, for example desloratadin [15], pseudoephedrine [16–20], montelukast [21], pseudoephedrin and dexbrompheniramine [22], and pseudoephedrine and ibuprofen [23]. Thin-layer chromatography (TLC) has been used for determination of loratadine either alone [9] or in the presence of impurities [14] or pseudoephedrine [19]. Although analysis of loratadine-containing pharmaceuticals has been described in numerous papers, no methods for simultaneous analysis of loratadine and preservatives are available in the literature. According to good manufacturing practice (GMP) regulations, active substances and preservatives used for human drug formulations should be analysed. This prompted us to develop and evaluate a TLC method for simultaneous analysis of loratadine and commonly used preservatives, i.e. sodium benzoate and a mixture of methylparaben and propylparaben, in commercial medicinal syrups.

EXPERIMENTAL

Chemicals and Reagents

Loratadine and preservatives (sodium benzoate, methylparaben, and propylparaben) were kindly provided by the Medicines and Medical Devices Agency of Serbia (Belgrade, Serbia). Other reagents used in the study were Merck (Darmstadt, Germany) products of analytical-grade purity.

Claritine syrup containing 1 mg loratadine per mL syrup and sodium benzoate (1 mg mL^{-1}) as preservative (Schering–Plough Pharmaceutical Works, Belgium) and Loratadine syrup containing the same amount of loratadine per mL and methylparaben (1 mg mL^{-1}) and propylparaben (0.1 mg mL^{-1}) as preservatives (Jugoremedia Pharmaceutical Works, Serbia) were analysed.

Placebo syrup was a solution of propylene glycol (4.5 mL), glycerol (1.5 mL), citric acid (1.5 mL of 5% aqueous solution), and saccharose (5 g) in water (25 mL).

Stock solutions of loratadine (1 mg mL^{-1}), methylparaben (1 mg mL^{-1}), and propylparaben (0.1 mg mL^{-1}) were prepared in ethanol and a stock solution of sodium benzoate (1 mg mL^{-1}) was prepared in 1:15 (*v*/*v*) acetic acid–ethanol. Standard solutions for calibration in the concentration ranges 0.15–0.35 mg mL^{-1} (loratadine, methylparaben and sodium benzoate) and 0.015–0.035 mg mL^{-1} (propylparaben) were prepared diluting the stock solutions with ethanol, so that application of 2 μL covered the ranges 0.3–0.7 μg per band for loratadine, methylparaben, and sodium benzoate, and 0.03–0.07 μg per band for propylparaben.

Analysis of Loratadine Syrup

Syrup (2.5 mL) was transferred to a 10-mL calibrated flask and diluted to volume with ethanol.

Chromatography

Chromatography was performed on 20 cm × 10 cm TLC plates cut from 20 cm × 20 cm aluminium plates precoated with silica gel 60 F_{254} (Merck). Standard and sample solutions (2.0 μL) were applied to the plates, 15 mm from the bottom, by means of a Camag (Muttenz, Switzerland) Nanomat II application device. Ascending chromatography to a distance of 95 mm was performed in a Camag twin-trough chamber. The mobile phases were *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*) (mobile phase S1) for mixtures of loratadine and sodium benzoate, and ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*) (mobile phase S2) for mixtures of loratadine, methylparaben, and propylparaben. After development the plates were dried in air and the zones were scanned in linear reflectance–absorbance mode at 240 nm (loratadine and sodium benzoate) or 275 nm (loratadine, methylparaben, and propylparaben) by means of a Camag TLC Scanner II with a computer system and Cats software (V.3.15). Peak areas were used for quantification.

RESULTS AND DISCUSSION

As is apparent from Fig. 1, the choice of *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*) as mobile phase enabled

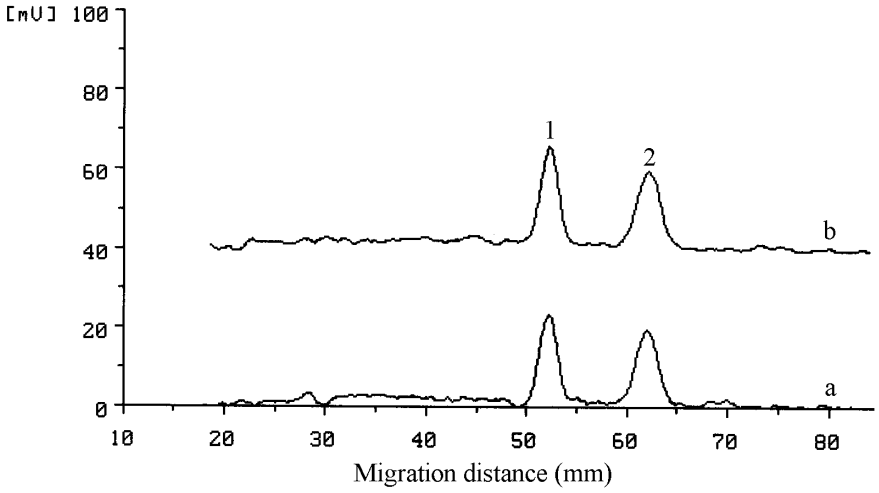

Fig. 1

Densitograms obtained from (a) Claritin syrup, and (b) loratadine (1) and sodium benzoate (2) standards. Mobile phase: *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*); wavelength 240 nm

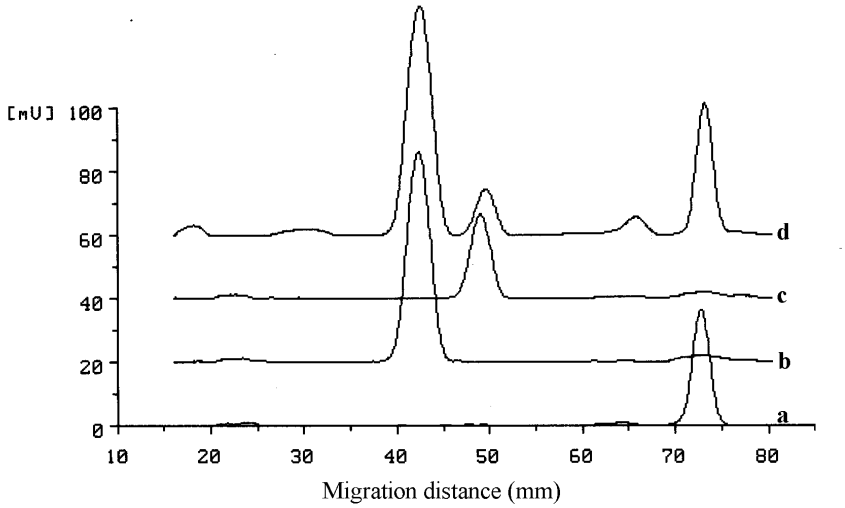

Fig. 2

Densitograms obtained from (a) loratadine, (b) methylparaben, (c) propylparaben, and (d) Loratadine syrup. Mobile phase: ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*); wavelength 275 nm

satisfactory resolution of loratadine (migration distance 52.5 mm, R_F 0.47) and sodium benzoate (migration distance 62.9 mm, R_F 0.60). Resolution was unaffected by other constituents of the syrup. Use of the same mobile phase for the mixture of loratadine, methylparaben, and propylparaben resulted in a poor resolution of the parabens, however, with R_F values close to 1. For this reason a new mobile phase, ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*) was optimized; the results are shown in Fig. 2. Application of this mobile phase resulted in greater mobility of loratadine (migration distance 70.5 mm, R_F 0.69) and lower mobility of the parabens (migration distances of methylparaben and propylparaben were 39.3 mm and 45.1 mm, respectively; R_F values 0.30 and 0.38). The identities of the bands of loratadine and the preservatives from syrups were confirmed by comparing the UV spectra of the separated bands with those of the standards ($r \geq 0.9997$). Peak purity of the analyte bands was assessed by comparing spectra acquired at the peak start (S), peak apex (M) and peak end (E) of the bands. Values of r(S,M) and r(M,E) ≥0.9998 showed that all analyte bands from the syrups were pure.

The wavelengths used for quantification of the individual components of the mixtures by TLC were chosen on the basis of the compounds' in-situ UV spectra. Loratadine, methylparaben, and propylparaben were quantified at 275 nm, i.e. at the absorption maximum of loratadine and where the absorbance of both methylparaben and propylparaben was quite high. Loratadine and sodium benzoate in the second mixture were quantified at 240 nm, at which both substances have similar absorbance. This wavelength was chosen because of very low absorbance of sodium benzoate within the wavelength range close to the absorption maximum of loratadine. In this way it was possible to quantify both components of the mixture in a single step.

Calibration plots were obtained within the concentration range 0.3–0.7 mg per band for loratadine, sodium benzoate, and methylparaben, and 0.03–0.07 mg per band for propylparaben. Relationships between peak area and amount for the chromatographed substances were obtained by linear regression analysis. Statistical data for calibration of each compound are listed in Table I.

All stock and calibration solutions were prepared in ethanol except for sodium benzoate stock solution which was prepared in acetic acid–ethanol 1:15 (*v*/*v*), because sodium benzoate was only slightly soluble in ethanol. Calibration solutions for sodium benzoate were obtained by diluting the stock solution with ethanol containing 0.6–2% acetic acid. For this reason

Table I

Statistical data for calibration plots obtained on the basis of peak area[a]

Substance	Mobile phase	Linear range (μg per zone)	Calibration function ($y = a + bx$)		
			$a \pm SD$	$b \pm SD$	r
Loratadine	S1[b]	0.300–0.700	165.4 ± 37.58	2006.5 ± 15.17	0.996
Sodium benzoate	S1	0.300–0.700	−35.46 ± 19.2	1476.6 ± 31.41	0.998
Loratadine	S2[c]	0.300–0.700	177.0 ± 12.9	1039.5 ± 27.2	0.997
Methylparaben	S2	0.300–0.700	764.3 ± 35.9	2328.9 ± 76.4	0.996
Propylparaben	S2	0.030–0.070	5.89 ± 6.91	5676.1 ± 116.2	0.997

[a]Calibration points were obtained in triplicate at five levels
[b]*n*-Butyl acetate–carbon tetrachloride–acetic acid–acetonitrile, 3:6:0.2:3 (*v*/*v*)
[c]Ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine, 1:4:0.8:0.4:2 (*v*/*v*)

the effects of different concentrations of acetic acid on R_F values and calibration data for sodium benzoate were examined. Two control series with the same sodium benzoate concentrations as in the calibration series were prepared. The concentration of acetic acid in all the solutions in the first series was 2% whereas that in solutions of the second series was twice as high as in the calibration solutions (i.e. 1.2–4%). The results obtained revealed that these different amounts of acetic acid had almost no effect on calibration data or R_F values for sodium benzoate.

The accuracy and precision of the method were established by analysis of a placebo syrup spiked with known amounts of loratadine and the preservatives. It is apparent from the data listed in Table II that relative standard deviations (*RSD*) range from 1.39% to 3.40%; this clearly shows the precision of the method is satisfactory.

The limits of detection (*LOD*) and quantification (*LOQ*) of loratadine and the preservatives were obtained experimentally on the basis of signal-to-noise ratios of 3:1 and 10:1, respectively (Table III).

The robustness of the method was studied by determining the effects of slight alteration of temperature, chamber saturation, chamber geometry, and development distance. Variation of the temperature between 22 and 28°C had no observable effect on separation of loratadine and the preservatives; at temperatures below 18°C, however, resolution of loratadine and sodium benzoate was poor. Chamber saturation had no effect on separation of the mixture of loratadine, methylparaben, and propylparaben but resulted in a lower R_F (0.53) for sodium benzoate. No significant changes of R_F were observed for any of the compounds when the effects of different chambers and development distances (85, 90, and 95 mm) were tested.

Table II

Accuracy and precision of the assay

Mixture	Substance	Spiked amount/zone (μg)	Found (μg)	*RSD*[a] (%)	Recovery[b] (%)
1[c]	Loratadine	0.400	0.405	2.02	101.2
		0.500	0.503	1.60	100.6
		0.600	0.585	2.31	98.7
	Sodium benzoate	0.400	0.391	1.98	98.3
		0.500	0.496	2.17	99.2
		0.600	0.618	2.42	101.9
2[d]	Loratadine	0.400	0.406	1.99	101.5
		0.500	0.499	2.02	99.8
		0.600	0.607	2.12	101.2
	Methylparaben	0.400	0.396	1.80	99.0
		0.500	0.503	1.39	100.6
		0.600	0.608	1.50	101.3
	Propylparaben	0.040	0.041	2.82	102.5
		0.050	0.051	3.40	102.0
		0.060	0.059	3.24	98.3

[a]$n = 3$
[b]Mean recovery
[c]Mobile phase: *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*)
[d]Mobile phase: ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*)

Table III

Limits of detection and quantification of loratadine and preservatives

Mixture	Substance	*LOD* (μg per zone)	*LOQ* (μg per zone)
1[a]	Loratadine	0.03	0.10
	Sodium benzoate	0.03	0.09
2[b]	Loratadine	0.01	0.04
	Methylparaben	0.004	0.01
	Propylparaben	0.004	0.01

[a]Mobile phase: *n*-butyl acetate-carbon tetrachloride-acetic acid-acetonitrile 3:6:0.2:3 (*v*/*v*)
[b]Mobile phase: ethyl acetate-*n*-hexane-methanol-ammonia-diethylamine 1:4:0.8:0.4:2 (*v*/*v*)

The method was used for determination of loratadine and sodium benzoate in Claritine syrup, and for determination of loratadine, methylparaben, and propylparaben in Loratadine syrup. The results obtained are listed in Table IV.

Table IV

Results from determination of loratadine and preservatives in commercial medicinal syrups

Syrup	Substance	Content (mg mL^{-1} syrup)	Percentage of label claim	*RSD*[a] (%)
Claritine[b]	Loratadine	0.9951	99.51	1.92
	Sodium benzoate	1.033	103.3	1.98
Loratadine[c]	Loratadine	0.9898	98.98	2.48
	Methylparaben	0.9982	99.82	1.80
	Propylparaben	0.0971	97.1	2.31

[a]$n = 6$
[b]Mobile phase: *n*-butyl acetate–carbon tetrachloride–acetic acid–acetonitrile 3:6:0.2:3 (*v*/*v*)
[c]Mobile phase: ethyl acetate–*n*-hexane–methanol–ammonia–diethylamine 1:4:0.8:0.4:2 (*v*/*v*)

CONCLUSIONS

This densitometric method for quantitative analysis of syrups containing loratadine, an antihistamine, as active agent, and sodium benzoate, methylparaben, and propylparaben as preservatives is accurate, simple, and rapid. It can be successfully used for simultaneous determination of loratadine and the preservatives in medicinal syrups. Preparation of syrup samples for analysis is simple and the method enables direct densitometric determination without previous isolation of the compounds of interest.

ACKNOWLEDGEMENT

This work was supported by the Ministry for Science and Environmental Protection of the Republic of Serbia, Grant # 142071.

REFERENCES

[1] D.T. Witiak, Antiallergenic agents, In A. Burger (ed.), Medicinal Chemistry, 3rd ed., New York, Wiley Interscience, 1970, p. 3.
[2] J.H. Block, J.M. Beale Jr., Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott Willians & Wilkins, eleventh ed., Philadelphia, 2004, p. 712.
[3] A.A. Gazy, H. Mahgoub, F.A. El-Yazbi, M.A. El-Sayed, and R.M. Youssef, J. Pharm. Biomed. Anal., **30**, 859 (2002)

[4] M.M. Ghoneim, M.M. Mabrouk, A.M. Hassanein, and A. Tawfik, J. Pharm. Biomed. Anal., **25**, 933 (2001)
[5] M. Nogowska, M. Zając, and I. Muszalska, Chem. Anal. (Warsaw), **45**, 681 (2000)
[6] N. El-Kousy and L. I. Bebawy, J. Pharm. Biomed. Anal., **20**, 671 (1999)
[7] P. Mikus, P. Kubacak, I. Valaskova, and E. Havranek, Pharmazie, **59**, 260 (2004)
[8] J.A. Squella, J.C. Sturm, M.A. Diaz, H. Pessoa, and L.J. Nunez-Vergara, Talanta, **43**, 2029 (1996)
[9] G. Indrayanto, L. Darmawan, S. Widjaja, and G. Noorrizka, J. Planar Chromatogr.-Mod. TLC, **12**, 261 (1999)
[10] S.J. Rajput and A.G. Vyas, Indian Drugs, **35,** 352 (1998)
[11] N.A. El Ragehy, A.M. Badawey, and S.Z.-El. Khateeb, J. Pharm. Biomed. Anal., **28**, 1041 (2002)
[12] H. Fernández, F.J. Rupérez, and C. Barbas, J. Pharm. Biomed. Anal., **31**, 499 (2003)
[13] F. J. Rupérez, H. Fernández, and C. Barbas, J. Pharm. Biomed. Anal., **29**, 35 (2002)
[14] N. Issa and M. Mlynarova, Ceska a Slovenska Farmacie, **53**, 192 (2004)
[15] D.T. El-Sherbiny, N. El-Enany, F.F. Belal, and S.H. Hansen, J. Pharm. Biomed. Anal., **43,** 1236 (2007)
[16] A. Abu-Lathou, I.I. Hamdan, and A. Tahraoui, Drug Dev. Ind. Pharm., **31**, 577 (2005)
[17] H. Mahgoub, A.A. Gazy, F.A. El-Yazbi, M.A. El-Sayed, and R.M. Youssef, J. Pharm. Biomed. Anal., **31**, 801 (2003)
[18] M.M. Mabrouk, H.M. El-Fatatry, S. Hammad, and A.A.M. Wahbi, J. Pharm. Biomed. Anal., **33**, 597 (2003)
[19] R.T. Sane, M. Francis, S. Khedkar, S. Pawar and A. Moghe, Indian Drugs, **38**, 436 (2001)
[20] G.V. Kanumula and B. Raman, Indian Drugs, **37**, 574 (2000)
[21] T. Radhakrishna, A. Narasaraju, M. Ramakrishna, and A. Satyanarayana, J. Pharm. Biomed. Anal., **31**, 359 (2003)
[22] F. Onur, C. Yucesoy, S. Dermis, M. Kartal, and G. Kokdil, Talanta, **51**, 269 (2000)
[23] D. Ivanović, M. Medenica, S. Marković, and G. Mandić, Arzneim.-Forsch., **50**, 1004 (2000)