ANALYSIS

IDENTIFICATION AND DETERMINATION OF SELECTED HISTAMINE ANTAGONISTS BY DENSITOMETRIC METHOD

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Abstract: The conditions for identification were determined for four histamine antagonists: clemastine fumarate, loratadine, cetirizine dihydrochloride and desloratadine by TLC (thin-layer chromatography) method. The selected chromatographic conditions were used to develop a densitometric method for the content determination of the histamine antagonists in medicinal products and substances. The statistical data showed adequate accuracy and precision of the developed methods.

Keywords: clemastine fumarate, loratadine, cetirizine dihydrochloride, desloratadine, histamine antagonist, densitometry.

The allergies are one of the four most common issues for public health along with tumors, cardiovascular diseases and AIDS. Each decade, a dramatic rise in allergies is observed in most countries.

A histamine antagonist is a pharmaceutical drug that inhibits allergy symptoms by blocking histamine receptors H_1.

Histamine antagonists competitively and reversibly bind H_1 receptors, inhibiting histamine as a mediator of inflammatory and immunological reactions. Histamine antagonists inhibit histamine reaction of virtually every smooth muscle.

In respect of their medical effect, two generations of this group with different pharmacokinetics and selectivity are available: standard first generation drugs and new second generation drugs (1, 2).

Cetirizine, loratadine, desloratadine and clemastine are histamine antagonists medicines routinely used in allergy treatment (Fig. 1). Therefore, these medicines are chosen for this study.

Clemastine (as clemastine fumarate) is the most commonly used histamine antagonist for sudden allergic reactions (1, 2).

Loratadine may be used to prevent allergic reaction of the respiratory tract and of the skin. Its active metabolite – desloratadine prolongs effect of the drug (2).

Cetirizine (as cetirizine dihydrochloride) is an active hydroxyzine metabolite. It is used in a symptomatic treatment of allergic reactions, i.e., hay fever, chronic rhinitis, urticaria, itch, and conjunctivitis (1, 2).

Desloratadine is an active loratadine metabolite. Desloratadine eliminates symptoms of hay fever, reduces asthma symptoms, and also has an antipruritic effect (2).

From the literature on the subject it can be concluded that in the last five years, clemastine fumarate was determined in tablets using spectrophotometric method (3) and in plasma using HPLC method with a mass spectrometry detector (4, 5).

Loratadine was determined in one-component tablets using HPLC method with UV detection (6) or in binary pharmaceutical forms with pseudoephedrine using spectrophotometric method (6, 7). HPLC method with a mass spectrometry detector (8, 9) was used for determining loratadine in plasma. Liquid chromatography LC (10–13) with C8 (10–12) or C18 (13) columns, UV detector (11, 13), mass spectrometry detector (12) or PDA detector (10) was used for determining cetirizine dihydrochloride. Also a capillary electrophoresis method (14) was used. Cetirizine dihydrochloride was deter-

Clemastine fumarate ($C_{25}H_{30}ClNO_5$, M.w. 459.97)
R-(R*,R*)-2-{2-[1-(4-Clorophenyl)-1-
phenylethoxy]ethyl}-1-methylpyrrolidine fumarate

Cetirizine ($C_{21}H_{25}ClN_2O_3$, M.w. 388.89
[2-[4-[(4-Chlorophenyl)-phenylmethyl]-
1- piperazinyl] ethoxy] acetic acid

Loratadine ($C_{22}H_{23}ClN_2O_3$, M.w. 382.88
4-(8-Chloro-5,6-dihydro-11H-
benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-
piperidinecarboxylic acid ethyl ester

Desloratidine ($C_{19}H_{19}ClN_2$, M.w. 310.82
8-Chloro-6,11-dihydro-11-(4-piperidinylidene-
5H-benzo[5,6]cyclohepta[1,2-b]pyridine

Figure 1. Compounds investigated

mined in combined tablets formulation (with pseudoephedrine) (10, 13) and in plasma as a single component (11) or simultaneously with pseudoephedrine (12).

HPLC or UPLC was the only method for determining desloratadine in a biological material (8, 15) and in medicinal products (6, 16, 17). Mass spectrometry detector (8, 15), spectrophotometric detector (6, 16) and PDA detector (17) were used. C18 (6, 8, 15, 16) columns and Aquity BEH C18 column for UPLC (17) were used. Desloratadine was determined simultaneously with loratadine in pharmaceutical preparation (6) or as an active metabolite in plasma (8).

Due to a high incidence rate of allergic reactions, histamine antagonists are a widely used group of compounds. Cost effective and easy methods for the determination are required as an alternative to liquid chromatography.

The purpose of the study was to develop a sensitive, simple and cost-effective TLC method for identification and determination of discussed compounds: clemastine fumarate, loratadine, cetirizine hydrochloride and desloratadine.

EXPERIMENTAL

Materials for analysis and instrumentation

Clemastine fumarate Ref. St. - Ph. Eur., active substance - Polfa Warszawa, Clemastinum 1 mg tablets - Polfa Warszawa.

Loratadine Ref. St. - Ph. Eur, active substance - Biotecpharma, Rotadin 10 mg tablets - Anpharm.

Cetirizine dihydrochloride Ref. St. - Ph. Eur, active substance - Egis Pharmaceutical, Cet Alergin 10 mg tablets - ICN Polfa Rezszów

Desloratadine Ref. St. - Schering Plough Ltd,, active substance - Schering Plough Ltd, Aerius 5 mg tablets - Schering Plough Ltd.

Analytically pure and high purity reagents for HPLC by Lab Scan, Merck HPTLC Silica gel 60 F_{254} and Merck HPTLC LiChrospher Silica gel 60 RP-18 WF_{254}s chromatographic glass plates, Hanau UV lamp, Camag automatic applicator, Shimadzu CS 9000 densitometer, Dionex *Ultimate 3000* liquid chromatograph or Shimadzu liquid chromatograph with *SPD-10 AVVP* spectrophotometric detector, *SCL-10 AVP* autosampler and *LC-10 AT VP* pump.

Qualitative analysis

Standard solutions: clemastine fumarate and loratadine in trichloromethane – methanol (1:1) as well as cetirizine dihydrochloride and desloratadine in methanol of the following concentrations: 1, 0.1 and 0.01 mg/mL.

Quantities of 30, 20, 10, 5, 2.5, 2, 1.5, 1, 0.5, 0.25, 0.2, 0.1, 0.05 and 0.025 µg of active substances were put as spots onto Merck HPTLC silica gel 60 F_{254} or Merck HPTLC LiChrospher silica gel 60 RP-18 WF_{254}s chromatographic plates (2 cm from the edge and 2 cm from the bottom) and developed in selected mobile phases up to 1 cm from the top edge. The following seven chromatographic systems were used:

1) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: trichloromethane – methanol – 25% ammonia (90:10:1, v/v/v) with chamber saturation;
2) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: diethyl ether – diethylamine (40:1, v/v);
3) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: methanol – ammonia 25% (100:1.5, v/v);
4) Merck HPTLC LiChrospher Silica gel 60 RP-18 WF_{254}s chromatographic plates and mobile phase: acetonitrile – methanol – acetate buffer at pH 5.5 (3:2:5, v/v/v);
5) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: toluene – ethyl acetate – 100% acetic acid (16:4:1, v/v/v);
6) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: n-hexane – acetone – 25% ammonia (85:14:1, v/v/v),
7) Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: acetonitrile – water – ammonia 25% (90:10:1, v/v/v).

The plates were air dried and the spot positions were determined in 254 nm UV light and developed in iodine vapors. Rf values were determined. Limits of detection (LOD) were established for all analyzed compounds by visual methods.

The results have been presented in Table 1.

Densitometric quantitative analysis

The following systems were used for determination of analyzed compounds: system II for clemastine fumarate and loratadine: Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: diethylether – diethylamine (40:1,v/v), system IV for cetirizine dihydrochloride: Merck HPTLC LiChrospher silica gel 60 RP-18 WF_{254}s chromatographic plates and mobile phase: acetoni-

Table 1. Rf values and limits of detection for the tested compounds (gray fields refer to the mobile phase selected for the quantitative determination of a given compound).

Tested compound		System 1 Rf	System 1 UV [µg]	System 1 iodine [µg]	System 2 Rf	System 2 UV [µg]	System 2 iodine [µg]	System 3 Rf	System 3 UV [µg]	System 3 iodine [µg]	System 4 Rf	System 4 UV [µg]	System 4 iodine [µg]	System 5 Rf	System 5 UV [µg]	System 5 iodine [µg]	System 6 Rf	System 6 UV [µg]	System 6 iodine [µg]	System 7 Rf	System 7 UV [µg]	System 7 iodine [µg]
Clemastine fumarate	Clemastine	0.78	1.5	0.05	0.55	1	-	0.58	2.5	0.1	0.12	2.5	2.5	0.0	0.5	0.5	0.16	2.5	0.1	0.48	2.5	0.1
	Fumaric acid	0.0	1.5	1.5	0.0	1.5	-	0.0	2.5	1	0.93	5	2.5	0.14	0.5	0.5	0.0	2.5	1	0.95	2.5	0.1
Loratadine		0.94	0.05	0.05	0.44	0.05	-	0.9	0.1	0.5	0.1	5	2.5	0.11	0.05	0.05	0.15	0.1	0.1	0.9	0.1	0.1
Cetirizine dihydrochloride		0.05	10	1	0	5	5	0.95	5	0.1	0.81	1	0.2	0	5	0.2	0	2	1	0.3	1	0.2
Desloratadine		0.26	0.05	0.2	0	0.1	10	0.28	0.1	0.5	0.26	0.05	0.2	0	0.1	0.2	0	0.1	0.2	0.1	0.1	0.2

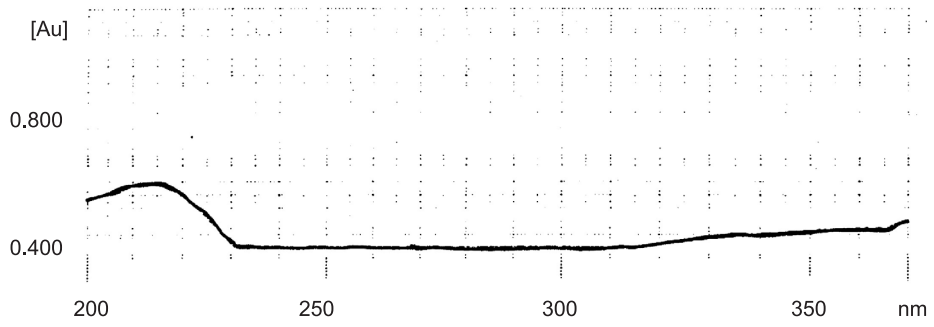Chromatographic systems numeration – see text.

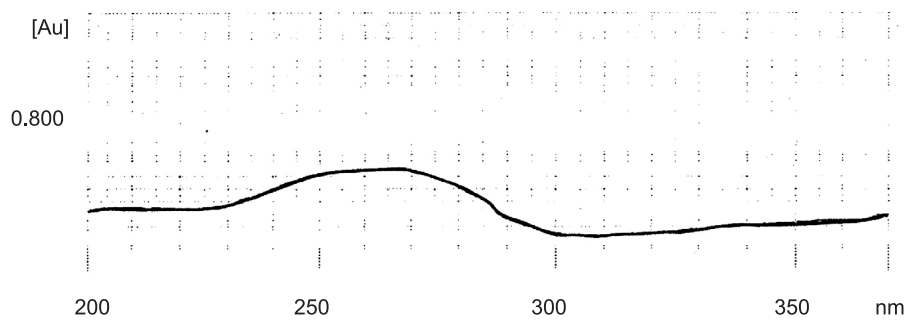Figure 2. UV spectrum of clemastine measured directly from the TLC plate

Figure 3. UV spectrum of loratadine measured directly from the TLC plate
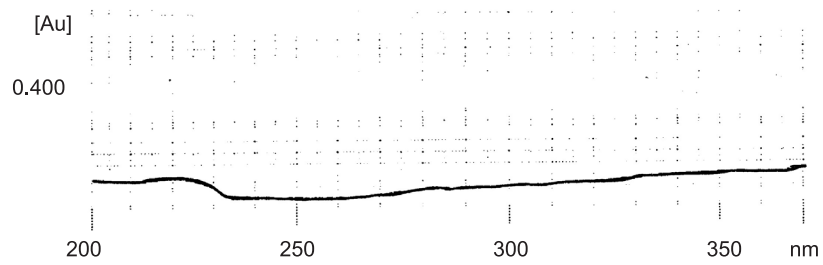
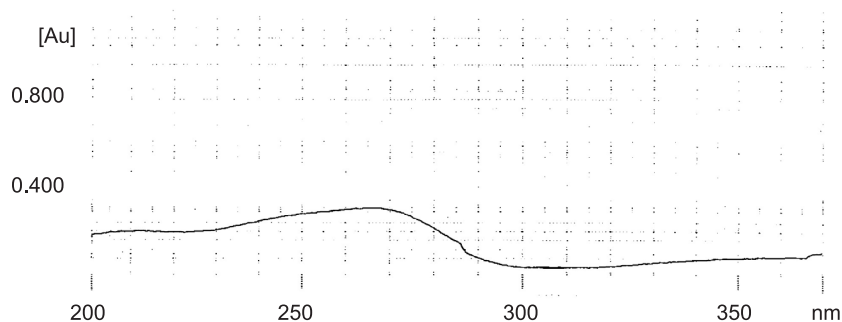Figure 4. UV spectrum of cetirizine measured directly from the TLC plate

Figure 5. UV spectrum of desloratadine measured directly from the TLC plate

trile – methanol – acetate buffer at pH 5.5 (3:2:5, v/v/v) and system I for desloratadine: Merck HPTLC silica gel 60 F_{254} chromatographic plates and mobile phase: trichloromethane – methanol – 25% ammonia (90:10:1, v/v/v) with chamber saturation.

Densitometric analyses were conducted using Shimadzu CS 9000 densitometer. Chromatographic plates were placed inside the chamber. Slit dimensions were 0.4 × 0.4 mm. Measurements were made by zigzag scanning with the following width of deflection: 12 mm for clemastine fumarate and desloratadine, 14 mm for loratadine, 16 mm for cetirizine dihydrochloride.

Maximum wavelengths were determined for all analyzed compounds: 215 nm for clemastine fumarate (Fig. 2), 260 nm for loratadine (Fig. 3), 220 nm for cetirizine dihydrochloride (Fig. 4) and 268 nm for desloratadine (Fig. 5).

Determination of regression curves

Solutions of standards, substances and medicinal products with following concentrations were prepared: 0.25, 0.5, 1, 2 and 3 mg/mL for clemastine fumarate and *Clemastinum tablets 1 mg* in trichloromethane – methanol (1:1, v/v); 0.1, 0.25, 0.5, 1 and 2 mg/mL for loratadine and *Rotadin tablets 10 mg* in trichloromethane – methanol (1:1, v/v); 0.5, 1, 2, 2.5, 3, 4 and 5 mg/mL for cetirizine dihydrochloride and *Cet Alergin tablets 10 mg* in methanol; 0.1, 0.2, 0.3, 0.5, 0.7 and 1 mg/mL for desloratadine and *Aerius tablets 10 mg* in methanol. All samples were shaken for 14 min in an ultrasonic bath and for 30 min in a mechanical shaker and

strained through 0.45 μm filters. Portions of 10 μL of prepared solutions were transferred onto chromatographic plates (2 cm from the edge and 2 cm from the bottom). After the development (up to 1 cm from the top plate edge), the plates were air dried and examined in 254 nm UV light and densitometric measurements were made at selected wavelengths determined prior to analysis.

The regression curves were determined for analyzed compounds, showing the linearity in the following range: 2.5–30 μg for clemastine fumarate, 1–20 μg for loratadine, 5–50 μg for cetirizine dihydrochloride and 1–10 μg for desloratadine.

Correlation coefficients R^2 were as follows: 0.9853 for clemastine fumarate, 0.9899 for loratadine, 0.9981 for cetirizine dihydrochloride and 0.998 for desloratadine. Equations of curves for specific compounds were as follows: y = 12763x + 54593 for clemastine fumarate, y = 21635x + 43580 for loratadine, y = 8229x + 17137 for cetirizine dihydrochloride and y = 15106x + 17307 for desloratadine.

Limits of quantification (LOQ) were determined on the basis of signal-to-noise (S/N) ratio (at an S/N of 10:1) and were as follows: 2.5 μg for clemastine fumarate, 5 μg for cetirizine dihydrochloride, 1 μg for loratadine and desloratadine. The statistical data obtained for developed densitometric methods and comparative methods are similar.

Quantitative analysis
Preparation of solutions
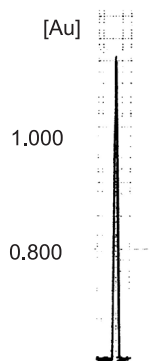The following concentrations of analyzed substances, standard substances and medicines were

[Au]
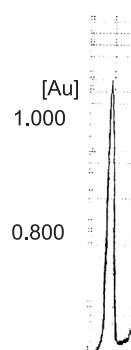
1.000

0.800

Figure 6. Densitogram of clemastine at 215 nm

[Au]
1.000

0.800

Figure 7. Densitogram of desloratadine at 268 nm
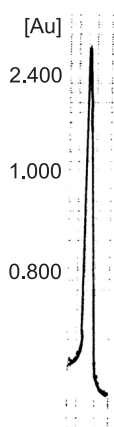
[Au]

2.400

1.000

0.800

Figure 8. Densitogram of loratadine at 260 nm
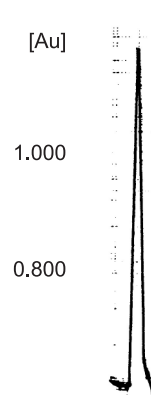
[Au]

1.000

0.800

Figure 9. Densitogram of cetirizine at 220 nm

prepared: 1 mg/mL for clemastine fumarate and 1 mg/mL for loratadine in trichloromethane – methanol (1:1, v/v), 2 mg/mL for cetirizine dihydrochloride and 0.5 mg/mL for desloratadine in methanol.

The samples were shaken for 14 min in an ultrasonic bath and for 30 min in a mechanical shaker. The solutions were strained through 0.45 μm filters.

Determination of the content

Several different mobile phases and different types of chromatographic plates were tested. The Merck HPTLC silica gel 60 F_{254} or Merck HPTLC LiChrospher silica gel 60 RP-18 $WF_{254}s$ chromatographic plates by Merck were chosen as stationary phases.

Of the seven chromatographic system tested, based of the results, system 2, 3 and 7 are the most

suitable for determination of clemastine fumarate. System 1 can be used to analyze clemastine's purity. System 2 is the most suitable for loratadine determination; system 3 and 5 are recommended to analyze loratadine's purity. System 4 and later system 3 and 7 are the most suitable for determination of cetirizine dihydrochloride. Systems 1, 2, 5 and 6 may be used to analyze cetirizine's purity. System 1, 3 or 4 may be used for determination of desloratadine, and system 2, 5 and 7 may be used to analyze purity of this component.

Rf values and broadening of chromatographic spots were taken into consideration when selecting system for quantitative analysis of specific components.

Finally, the following systems were used for quantitative analysis: system 2 for clemastine fumarate and loratadine (Rf = ca. 0.5 for both compounds), system 4 for cetirizine dihydrochloride (Rf

Table 2. Statistical of the determination of the tested compounds in pharmaceutical substances.

Tested compound	Method	Number of samples	Arithmetic mean of all measurements X [%]	Standard deviation S [%]	Confidence interval X ± ΔX PU = 95%[%]	RSD [%]
Clemastine fumarate	Densitometric	6	99.94	0.33	99.94 ± 0.31	0.33
	HPLC	6	100.14	0.34	100.14 ± 0.33	0.34
Loratadine	Densitometric	6	99.64	0.35	99.64 ± 0.34	0.36
	HPLC	6	100.42	0.29	100.42 ± 0.28	0.29
Cetirizine dihydrochloride	Densitometric d	8	99.43	0.45	99.43 ± 0.62	0.45
	HPLC	8	99.84	0.20	99.84 ± 0.29	0.20
Desloratadine	Densitometric	8	100.58	0.25	100.58 ± 0.35	0.25
	HPLC	8	100.28	0.33	100.28 ± 0.47	0.33

Table 3. Statistical assessment of the results concerning the determination of the tested compounds in pharmaceutical products.

Tested compound	Method	Number of samples	Arithmetic mean of all measurements X [mg]	Standard deviation S [mg]	Confidence interval X ± ΔX PU = 95% [mg]	RSD [%]
Clemastinum tablets 1 mg (Clemastine fumarate)	Densitometric	6	0.92	0.01	0.92 ± 0.01	1.13
	HPLC	6	0.94	0.01	0.94 ± 0.01	1.41
Rotadin tablets 10 mg (Loratadine)	Densitometric	6	10.00	0.08	10.00 ± 0.08	0.85
	HPLC	6	9.91	0.04	9.91 ± 0.04	0.44
Cet Alergin tablets 10 mg (Cetirizine dihydrochloride)	Densitometric	8	10.37	0.07	10.37 ± 0.10	0.45
	HPLC	8	10.32	0.09	10.32 ± 0.13	0.90
Aerius tablets 5 mg (Desloratadine)	Densitometric	8	4.82	0.04	4.82 ± 0.05	0.81
	HPLC	8	4.90	0.02	4.90 ± 0.03	0.47

= 0.81) and system 1 for desloratadine (Rf = 0.26). Automatic application was used to ensure the highest precision of the densitometric method.

Conformity of spectra for analyzed substances, drugs and standard substances was demonstrated and linearity for all analyzed compounds was observed.

Cetirizine dihydrochloride, clemastine fumarte and desloratadine: a 10 µL portions of standard solutions and prepared solutions were applied on Merck HPTLC LiChrospher silica gel 60 RP-18 WF$_{254}$S chromatographic plates.

Loratadine: a 5 µL portion of standard solutions and prepared solutions were transferred on Merck HPTLC silica gel 60 F$_{254}$ chromatographic plates.

After the development (up to 1 cm from the top plate edge), the plates were air dried and densitometric measurements at the selected wavelengths were made (Fig. 6–9).

Simultaneously, determination of tested compounds in medicinal products and substances using reference methods was conducted. HPLC method specified in the USP was applied for determining clemastine fumarate, loratadine and cetirizine dihydrochloride. A method described by El-Sherbing et al. was applied for determining desloratadine (6).

The results and statistical assessment are presented in Tables 2 and 3.

CONCLUSIONS

A comparison of the results of quantitative determination of the four histamine antagonists showed that the differences in the results of the HPLC and TLC methods are statistically insignificant.

The developed densitometric methods are simple, fast and cost-effective, and may have wide applications in the analysis of discussed compounds.

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