ANALYSIS

IDENTIFICATION AND DETERMINATION OF KETOTIFEN HYDROGEN FUMARATE, AZELASTINE HYDROCHLORIDE, DIMETINDENE MALEATE AND PROMETHAZINE HYDROCHLORIDE BY DENSITOMETRIC METHOD

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Abstract: Conditions for determination of: ketotifen hydrogen fumarate, azelastine hydrochloride, dimetindene maleate and promethazine hydrochloride by densitometric method in substances and pharmaceuticals were provided. Maximum wavelenghts were: 228 nm for ketotifen hydrogen fumarate, 295 nm for azelastine hydrochloride, 265 nm for dimetindene maleate and 255 nm for promethazine hydrochloride. The limits of quantification were in the ranges of 0.2–5 µg/spot. The statistical data showed adequate accuracy and precision of developed methods.

Keywords: ketotifen hydrogen fumarate, azelastine hydrochloride, dimetindene maleate, promethazine hydrochloride, histamine antagonist, densitometry

Allergy means all excessive reactions of healthy people to neutral substances.

Histamine is a physiologically active, endogenous substance that activates H_1 and H_2 receptors. It is responsible for allergic reaction in our body.

The mechanism of action of antihistamines is based on their competitive and reversible connection between H_1 and H_2 receptors. It annihilates effects of histamine as a mediator of inflammation and immune response (1, 2).

Ketotifen hydrogen fumarate, azelastine hydrochloride, dimetindene maleate and promethazine hydrochloride are histamine antagonists medicines usually used in allergy treatment. Therefore, these medicines were chosen for this study.
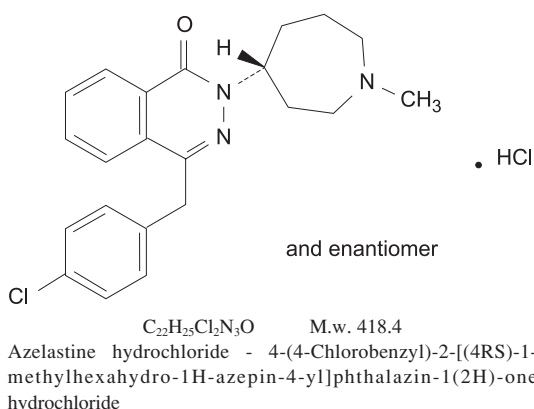
Ketotifen (as hydrogen fumarate) is able to inhibit the release of histamine, other relay basophils and mast cells. It causes long-lasting inhibition of histamine reactions. It is effective in the treatment of allergic diseases as well as asthma. It is used to prevent asthma attacks caused by allergy, it also works antianafilactically (1, 2).
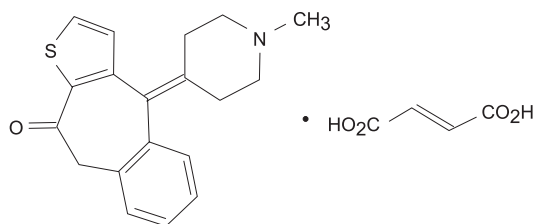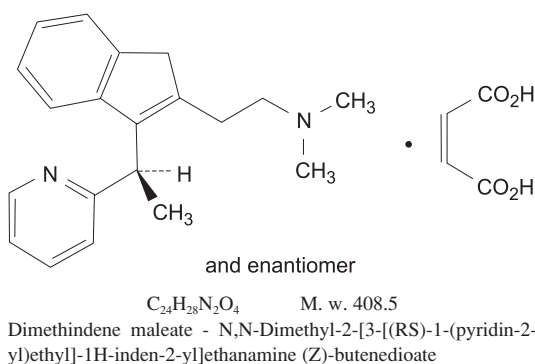

$C_{23}H_{23}NO_5 S$ · · · · · M.w. 425.5

Ketotifen hydrogen fumarate - 4-(1-Methylpiperidin-4-ylidene)-4,9-dihydro-10H-benzo[4,5]cyclohepta[1,2-b]thiophen-10-one hydrogen (E)-butenedioate

Azelastine (as hydrochloride) is a II generation antihistamine drug. Not only does it block receptors for H_1, but also inhibits the release of histamine. Following oral administration, it is metabolized and a major metabolite, which is N-demethylazelastine, shows properties of antihistamines. It is used in symptomatic treatment of allergies and as an adjunct in treatment of bronchial asthma (1).
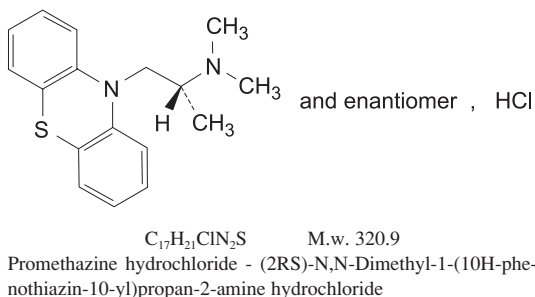
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$C_{22}H_{25}Cl_2N_3O$ M.w. 418.4

Azelastine hydrochloride - 4-(4-Chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl]phthalazin-1(2H)-one hydrochloride

Dimetindene (as maleate) is a I generation antihistamine. It shows a very strong antipruritic activity. It is administered orally: retard tablet (every 12 h) or drops and topically on the skin as a gel (1, 2).

$C_{24}H_{28}N_2O_4$ M. w. 408.5

Dimethindene maleate - N,N-Dimethyl-2-[3-[(RS)-1-(pyridin-2-yl)ethyl]-1H-inden-2-yl]ethanamine (Z)-butenedioate

Promethazine (as hydrochloride) is a phenothiazine derivative of commonly used, slow, but long-acting antihistaminic. It also has hypnotic properties, antiemetic, anticholinergic and analgesic. Promethazine as therapeutic doesn't affect cardiovascular system, neither does it work as psychotic or neuroleptic (1, 2).

$C_{17}H_{21}ClN_2S$ M.w. 320.9

Promethazine hydrochloride - (2RS)-N,N-Dimethyl-1-(10H-phenothiazin-10-yl)propan-2-amine hydrochloride

From the literature on the subject it can be concluded that during last years, ketotifen hydrogen fumarate was determined in tablets using

chemiluminescence method (3) and in plasma using HPLC method with a mass spectrometry detector (4).

Azelastine, its enantiomers and metabolites were determined in rats plasma using coupling HPLC method with a β-cyclodextrin chiral stationary phase to ion-spray tandem mass spectrometry. Additionally, an enatioselective assay was compared to another assay using electrokinetic capillary chromatography with β-cyclodextrin and carboxymethyl-β-cyclodextrin in polyacrylamide-coated capillaries (5). Moreover, HPLC method, with a mass spectrometry detector and chiral column, was used to determine azelastine in plasma (6).

Liquid chromatography (LC) with ZIC-HILIC column and UV detector was used to determine dimetindene maleate in gel (7). Capillary electrophoresies (CE) with a diode array detection (DAD) was used to determine dimetindene binding to human plasma proteins (8).

Promethazine hydrochloride was determined in medicinal products (9–14) using spectrophotometric method (9, 10), capillary electrophoresis (11), densitometry (12), portable system of programmable syringe pump with potentiometer (13) and highly accurate nephelometric titration (14). Voltammetric method with carbon paste electrode (15) and HPLC-ESI-MS (16) were used to determine promethazine in biological material.

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: ketotifen hydrogen fumarate, azelastine hydrochloride, dimetindene maleate and promethazine hydrochloride.

EXPERIMENTAL

Reference materials

Ketotifen hydrogen fumarate (Novartis), Azelastine hydrochloride (Ph. Eur.), Dimetindene maleate (Ph. Eur.), Promethazine hydrochloride (Ph. Eur.).

Active substances

Ketotifen hydrogen fumarate (Novartis), Azelastine hydrochloride (Meda Pharma), Dimetindene maleate (Novartis).

Medicinal products

Ketotifen 1 mg tablets (Polfa Warszawa), Allergodil 0.1% solution (Meda Pharma), Fenistil 1 mg/g gel (Novartis), Diphergan 25 mg dragées (Jelfa).

Reagents and apparatus

Analytically pure and high purity reagents for HPLC from 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 of ketotifen hydrogen fumarate, azelastine hydrochloride, dimetindene maleate and promethazine hydrochloride in methanol of the following concentrations: 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL were prepared.

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 - ammonia 25% (90 : 10 : 1, v/v/v) with chamber saturation;

2. Merck HPTLC Silica gel 60 F_{254} chromatographic plates and mobile phase: diethylether – 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 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 – acetic acid 100% (16 : 4 : 1, v/v/v);

6. Merck HPTLC Silica gel 60 F_{254} chromatographic plates and mobile phase: n-hexane – acetone – ammonia 25% (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 spots positions were determined under 254 nm UV light and in iodine vapors. Rf values were determined. Limits of detection (LOD) were established for all analyzed compounds by visual methods.
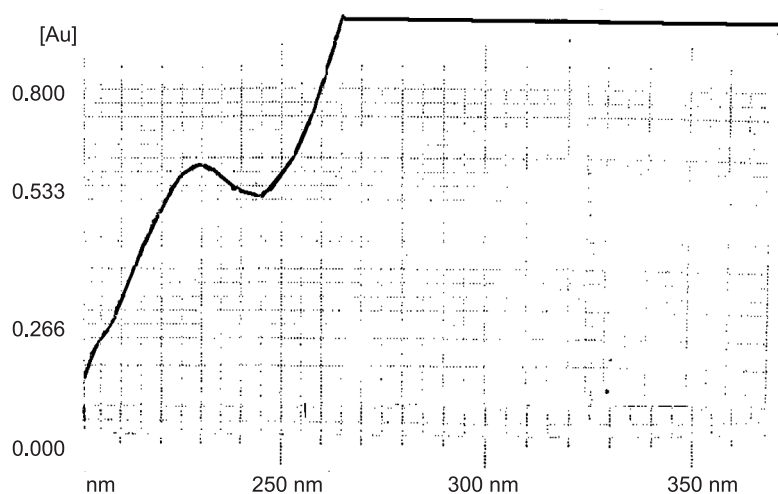

Figure 1. UV spectrum of ketotifen hydrogen fumarate measured directly from the TLC plate

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			System 2			System 3			System 4			System 5			System 6			System 7		
	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]	Rf	Limit of detection [µg/spot] UV [µg]	iodine [µg]
Ketotifen hydrogen fumarate	0.96	0.25	0.1	0.34	0.25	-	0.58	0.25	0.25	0.15	0.1	0.25	0.02	0.25	0.25	0	0.25	0.25	0.5	0.25	0.25
Azelastine hydrochloride	0.54	0.2	0.1	0.28	0.1	0.5	0.49	0.1	0.05	0.5	0.1	0.1	0	0.1	0.1	0	0.25	0.25	0.36	0.1	0.1
Dimetindene maleate	0.64	0.05	0.1	0.28	0.02	0.1	0.51	0.1	0.1	0.26	0.1	0.2	0	0.1	0.2	0	0.1	0.2	0.26	0.1	0.2
Promethazine hydrochloride	0.96	0.1	0.1	0.44	0.05	-	0.62	0.1	0.25	0.09	0.1	0.25	0.06	0.1	0.25	0.16	0.1	0.25	0.6	0.15	0.25

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

The results are presented in Table 1.

Densitometric quantitative analysis

The following systems were used for determination of analyzed compounds: system II for azelastine hydrochloride, dimetindene maleate and promethazine hydrochloride: Merck HPTLC Silica gel 60 F_{254} chromatographic plates and mobile phase: diethylether – diethylamine (40 : 1, v/v); system III for ketotifen hydrogen fumarate: Merck HPTLC Silica gel 60 F_{254} chromatographic plates and mobile phase: methanol – ammonia 25% (100 : 1.5, v/v). 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: 10 mm for azelastine hydrochloride, 12 mm for ketotifen hydrogen fumarate and dimetindene maleate, 14 mm for promethazine hydrochloride.
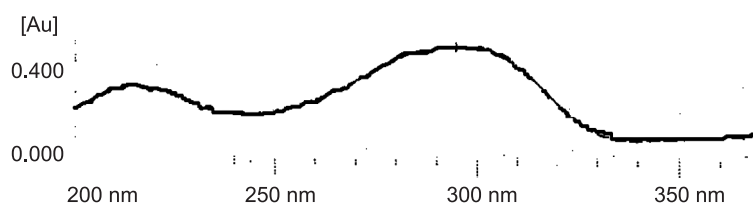
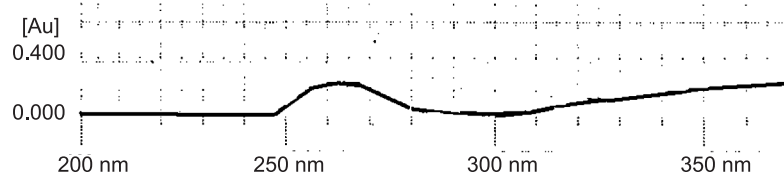Figure 2. UV spectrum of azelastine hydrochloride measured directly from the TLC plate

Figure 3. UV spectrum of dimetindene maleate measured directly from the TLC plate
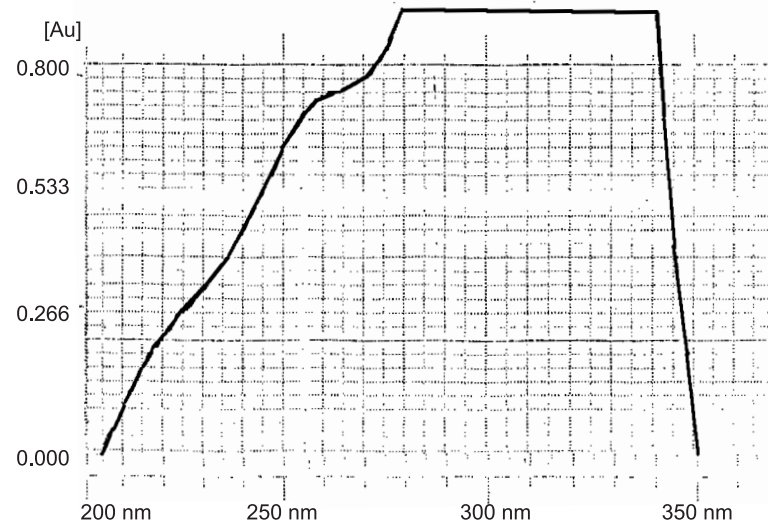
Figure 4. UV spectrum of promethazine hydrochloride measured directly from the TLC plate

Table 2. Statistical results 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 [%]
Ketotifen hydrogen fumarate	Densitometric method	6	100.38	0.14	100.38 ± 0.14	0.14
	Titration method in anhydrous medium	6	100.33	0.11	100.33 ± 0.10	0.10
Azelastine hydrochloride	Densitometric method	7	99.70	0.31	99.70 ± 0.44	0.31
	Titration method in anhydrous medium	7	99.78	0.30	99.78 ± 0.33	0.28
Dimetindene maleate	Densitometric method	7	100.18	0.61	100.18 ± 0.66	0.61
	Titration method in anhydrous medium	7	100.73	0.16	100.73 ± 0.17	0.16

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	Standard deviation S	Confidence interval X ± ΔX PU = 95%	RSD [%]
Ketotifen 1 mg tablets (Ketotifen hydrogen fumarate)	Densitometric method	6	0.97 mg	0.01	0.97 mg ± 0.01 mg	1.28
	HPLC	6	0.98 mg	0.01	0.98 mg ± 0.01 mg	1.41
Allergodil 0.1% solution (Azelastine hydrochloride)	Densitometric method	7	1.00 mg/mL	0.01	1.00 mg/mL ± 0.01 mg	0.65
	HPLC	7	0.99 mg/mL	0.00	0.99 mg/mL ± 0.00 mg	0.28
Fenistil 1 mg/g gel (Dimetindene maleate)	Densitometric method	7	0.97 mg/g	0.01	0.97 mg/g ± 0.02 mg	1.60
	HPLC	7	0.95 mg/g	0.01	0.95 mg/g ± 0.01 mg	1.16
Diphergan 25mg dragées (Promethazine hydrochloride)	Densitometric method	6	25.83 mg	0.08	25.83 mg ± 0.10 mg	0.32
	HPLC	6	25.95 mg	0.54	25.95 mg ± 0.52 mg	2.10

Maximum wavelengths were determined for all analyzed compounds: 228 nm for ketotifen hydrogen fumarate (Fig. 1), 295 nm for azelastine hydrochloride (Fig. 2), 265 nm for dimetindene maleate (Fig. 3) and 255 nm for promethazine hydrochloride (Fig. 4).

Determination of regression curves

Solutions of standards, substances (excluding promethazine hydrochloride, as authors do not have access to this substance) and medicinal products with following concentrations were prepared in methanol: 0.5, 0.75, 1, 1.25 and 1.5 mg/mL for ketotifen hydrogen fumarate and Ketotifen 1 mg tablets; 0.02, 0.05, 0.1, 0.15, 0.25, 0.4 and 0.5 mg/mL for azelastine hydrochloride but Allergodil 0.1% solution was transferred directly from container; 0.05,

0.1, 0.2, 0.3, 0.5 and 0.7 mg/mL for dimetindene maleate and Fenistil 1 mg/g gel; 0.25, 0.5, 0.75, 1 and 1.25 mg/mL for promethazine hydrochloride and Diphergan 25 mg dragées.

All samples (excluding Allergodil 0.1% solution) 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 mL of prepared solutions were transferred onto chromatographic plates (2 cm from the edge and 2 cm from the bottom) and portions of: 0.2, 0.5, 1, 1.5, 2.5, 4 and 5 μL were transferred directly from container of Allergodil 0.1% solution. 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.
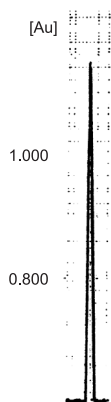

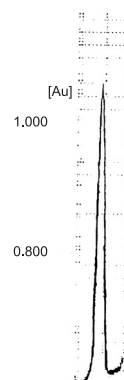Figure 5. Densitogram of ketotifen hydrogen fumarate at 228 nm

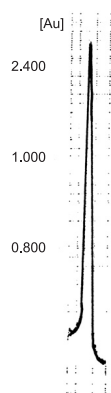Figure 6. Densitogram of azelasine hydrochloride at 295 nm

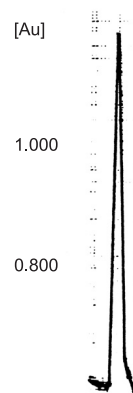Figure 7. Densitogram of dimetindene maleate at 265 nm

Figure 8. Densitogram of promethazine hydrochloride at 255 nm

The regression curves were determined for analyzed compounds, showing the linearity in the following range: 5-15 μg/spot for ketotifen hydrogen fumarate, 0.2-5 μg/spot for azelastine hydrochloride, 0.5-7 μg/spot for dimetindene maleate, 2.5-12.5 μg/spot for promethazine hydrochloride.

Correlation coefficients R were as follows: 0.9955 for ketotifen hydrogen fumarate, 0.9943 for azelastine hydrochloride, 0.9937 for dimetindene maleate, and 0.9925 for promethazine hydrochloride. Equations of a curve for specific compounds were as follows: $y = 9589.6x + 62560$ for ketotifen hydrogen fumarate, $y = 21475x + 12704$ for azelastine hydrochloride, $y = 17727x + 15920$ for dimetindene maleate, $y = 18018x + 95204$ for promethazine hydrochloride.

The 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: 5 mg/spot for ketotifen hydrogen fumarate, 0.2 μg/spot for azelastine hydrochloride, 0.5 μg/spot for dimetindene maleate and 2.5 μg/spot for promethazine hydrochloride. 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 (excluding Allergodil 0.1% solution which was transferred directly from container) were prepared in methanol: 1 mg/mL for ketotifen hydrogen fumarate, promethazine hydrochloride and azelastine hydrochloride and 0.1 mg/mL for dimetindene maleate.

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, systems 2, 3 and 7 are the most suitable for determination of ketotifen hydrogen fumarate and promethazine hydrochloride. Other systems can be used to analyze these substances's purity. Systems 1, 3 and 4 are the most suitable for azelastine hydrochloride determination; system 5 is recommended to analyze azelastine's purity. Systems 1 and 3 are the most suitable for determination of dimetindene maleate. Systems 5 and 6 may be used to analyze dimetindene's purity.

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 azelastine hydrochloride, dimetindene maleate (Rf = ca. 0.3 for both compounds) and promethazine hydrochloride (Rf = ca. 0.4), system 3 for ketotifen hydrogen fumarate (Rf = ca. 0.6).

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.

Ketotifen hydrogen fumarate: a 10 μL portion of standard solutions and prepared solutions were applied on Merck HPTLC Silica gel 60 F_{254} chromatographic plates.

Azelastine hydrochloride: a 1.5 μL portion of standard solutions, prepared solutions and Allergodil 0.1% solution (directly from container) were transferred into Merck HPTLC Silica gel 60 F_{254} chromatographic plates.

Dimetindene maleate: a 20 μL portion of standard solutions and prepared solutions were transferred into Merck HPTLC Silica gel 60 F_{254} chromatographic plates.

Promethazine hydrochloride: a 5 μL portion of standard solutions and prepared solutions were transferred into 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 (Figs. 5–8).

Simultaneously, determination of tested compounds in medicinal products and substances using reference methods (described in Ph. Eur.) was conducted. The titration method in anhydrous medium was applied for determination of all substances. HPLC (described for the determination of impurities) method was used for determination in medicinal products.

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

CONCLUSIONS

The developed densitometric methods are simple, fast and cost-effective.

The comparative methods of the results (HPLC for products and titration method in anhydrous medium for substances) showed that the deviations in the results are statistically insignificant. New methods may be widely applied or analysis of tested compounds.

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