There has been a significant increase in various allergic disorders over the last few decades. The essential medications used for the treatment of allergies are antihistamines.

The mechanism of action of antihistamines is based on their competitive and reversible binding to the H1 receptor that results in the abolition of the effects of histamine as a possible chemical mediator implicated in inflammation and allergic reactions.

In general, two groups of antihistamines can be distinguished: first generation and second generation types.

First generation H1-antihistamines have poor H1 receptor selectivity, and thus they may also block other receptors causing a number of side effects such as dryness of mucous membranes, arrhythmia, urination disorders, tachycardia, dizziness, increased appetite. In addition, they have the ability to cross the blood-brain barrier by inhibiting the physiological effects of histamine, and leading to drowsiness, visual disturbances, memory deficits and cognitive disorders.

In modern medicine, second-generation antihistamines play a fundamental role in the treatment of allergic disorders. Second generation H1-antihistamines are highly selective for the histamine H1 receptor and do not cross the blood-brain barrier (or cross only to a small extent). Second generation antihistamines differ from first generation because of their high selectivity and the lack of effect on the central nervous system (CNS). Therefore, second generation antihistamines exerted much less side effects compared with first generation antihistamines.

Modern second generation antihistamines (which are often metabolites or isomers of older drugs such as desloratadine – a product of loratadine metabolic transformation, or fexofenadine – a product of terfenadine metabolic transformation) also exhibit non-receptor anti-inflammatory effects (e.g., desloratadine and rupatadine) (1).

Rupatadine (Fig. 1) is a novel antihistamine of the second generation that was introduced into Europe for the treatment of allergic disorders. Its properties go beyond the characteristics of other drugs that belong to the group of second generation antihistamines. One moiety of this molecule has high affinity to H1 receptor, and the second one blocks the receptor for PAF (platelet activating factor). Rupatadine is able to work against both early-
and late-stage allergic reactions. In the early stage - through the antihistamine activity, in the later - through both the anti-inflammatory and anti-PAF effects.

Rupatadine is safe, not cardiotoxic and does not impair psychomotor or cognitive activity. The drug is well tolerated, with mild and moderate side effects. Rupatadine is highly effective in the treatment of allergic rhinitis, conjunctivitis and urticaria (1).

Fexofenadine (Fig. 2) is a modern second generation antihistamine. This antihistaminic drug potently and selectively blocks histamine H1 receptors, thus it has strong anti-allergic properties. Fexofenadine penetrates into CNS to a small extent, and does not cause sedation and drowsiness as side effects. It is used for the symptomatic treatment of allergic rhinitis and chronic idiopathic urticaria (2).

A review of the literature of the last five years reveals that rupatadine was determined in tablets with the following methods: spectrophotometric method at 244 nm (3), spectrophotometric method after conversion into rupatadine derivatives at 416, 511 and 527 nm (4) and HPLC method with UV detection on column C18 with a mixture of methanol – sodium phosphate monobasic – acetonitrile as mobile phase (5).

Fexofenadine was determined by a spectrophotometric method after conversion to fexofenadine derivatives at 612 and 615 nm (6), spectrophotometric method using the first derivative (7), spectrofluorometric method (fluorescence intensity was measured at 612 and 615 nm (8), HPLC method performed on a Zorbax Eclipse XDB-C8 (9, 10) column with a mixture of ammonium acetate – formic acid – methanol (1 mmol/L) as mobile phase and MS detector (9), or with a mixture of phosphate buffer – acetonitrile – methanol (1 mmol/L) as mobile phase and UV detector (10), and UPLC method, in gradient (with the following mobile phases: 0.1% formic acid and 1% formic acid with acetonitrile) performed on a column C18 with UV detection (11). Fexofenadine was determined in medicinal products (6-8, 10) and in plasma (9, 11).

Antihistamines are currently a widely used group of drugs. Therefore, an attempt was made to develop simple and inexpensive methods of their determination.

Several methods for the identification (TLC) and densitometric methods for the determination of antihistamines: loratadine, clemastine, promethazine, ketotifen, cetirizine, desloratadine, dimetindene and azelastine (12, 13) were developed in past years at the Department of Basic and Applied Pharmacy of National Medicines Institute.

The aim of this study was to develop a sensitive, simple and cost-effective methods for the determination of two modern antihistaminic drugs, rupatadine and fexofenadine.
Identification and determination of rupatadine and fexofenadine by...

EXPERIMENTAL

Reagents and equipment
- analytically pure and high purity reagents for HPLC by Rathburn;
- chromatographic plates: HPTLC silica gel F254 and HPTLC silica gel RP18 F254s by Merck;
- Hanau UV lamp;
- Camag Automatic TLC sampler;
- Shimadzu CS-9000 scanning densitometer;
- liquid chromatograph Dionex Ultimate 3000 with a UV detector.

Materials
Reference materials:
- Rupatadine fumarate Ref. St. batch RUPAF-0910
- Fexofenadine hydrochloride Ref. St. batch 1.0
Tested substances:
- Rupafin, 10 mg tablets (Rupatadine fumarate), J.Uriach & Cia, S.A.
- Telfast 120, 120 mg film-coated tablets (Fexofenadine hydrochloride), Aventis

QUALITATIVE ANALYSIS

Identification of rupatadine fumarate and fexofenadine
At the first stage of the studies, optimal chromatographic systems were investigated for the identification of examined substances, rupatadine and fexofenadine.

Methanolic standard solutions of rupatadine fumarate and fexofenadine were prepared at the following concentrations: 1, 0.1 and 0.01 mg/mL.

The following amounts: 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...
examined substances were spotted on Merck silica gel F_{254} HPTLC or Merck silica gel RP 18 F_{254S} HPTLC plates. The chromatograms were then developed with various mobile phases. After air drying, the positions of the spots were examined under UV light at 254 nm and visualized in iodine vapor.

Several mobile phases for the qualitative analysis have been examined and the following seven HPTLC systems were selected:

I. Merck silica gel F_{254} HPTLC plates and mobile phase: chloroform – methanol – 25% ammonia (90 : 10 : 1, v/v/v), with chamber saturation;
II. Merck silica gel F_{254} HPTLC plates and mobile phase: diethyl ether – diethylamine (40 : 1, v/v);
III. Merck silica gel F_{254} HPTLC plates and mobile phase: methanol – 25% ammonia (100 : 1.5, v/v);
IV. Merck silica gel RP 18 F_{254S} HPTLC plates and mobile phase: acetonitrile – methanol – acetate buffer at pH 5.5 (3 : 2 : 5, v/v/v);
V. Merck silica gel F_{254} HPTLC plates and mobile phase: toluene – ethyl acetate – acetic acid (16 : 4 : 1, v/v/v);
VI. Merck silica gel F_{254} HPTLC plates and mobile phase: n-heksane – acetone – 25% ammonia (85 : 14 : 1, v/v/v);
VII. Merck silica gel F_{254} HPTLC plates and mobile phase: acetonitrile – water – 25% ammonia (90 : 10 : 1, v/v/v).

In qualitative analysis, the Rf values were calculated and the limits of detection of examined compounds were determined.

Obtained results are given in Table 1.

### QUANTITATIVE ANALYSIS

#### Determination of rupatadine fumarate

The densitometric method was used for the determination of rupatadine fumarate.

Two systems were selected for further testing. System VII (described in section considering identification of rupatadine): Merck silica gel F_{254} HPTLC plates and mobile phase: acetonitrile – water – 25% ammonia (90 : 10 : 1, v/v/v) was selected for the quantitative determination of rupatadine, and system IV (described in section considering identification of fexofenadine): Merck silica gel F_{254} HPTLC plates and mobile phase: acetonitrile – methanol – acetate buffer at pH 5.5 (3 : 2 : 5, v/v/v) was selected for the determination of fexofenadine. The maximum wavelengths determined were 256 nm and 210 nm for rupatadine and fexofenadine, respectively (Fig. 1 and Fig. 3).
Identification and determination of rupatadine and fexofenadine by...

All densitometric measurements were done with the Shimadzu CS 9000 densitometer. Chromatographic plates were placed within the densitometer chamber. The slit dimension was kept at 0.4 × 0.4 mm. Densitometric scanning was performed in a zigzag mode, while the width of deflection was 10 and 16 mm for rupatadine and fexofenadine, respectively (Fig. 2 and Fig. 4).

Determination of regression curves

In order to perform a regression analysis the solutions of standards and tablet samples were prepared.

The standard solutions of rupatadine fumarate and sample solutions obtained from Rupafin 10 mg tablets of 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 mg/mL were prepared in methanol.

The standard solutions of fexofenadine and sample solutions obtained from Telfast 120 mg tablets of 0.5, 1.0, 2.5, 4, 5 and 6 mg/mL were prepared in methanol.

The samples were shaken for 14 min into an ultrasonic bath, then, they were shaken for 30 min in a mechanical shaker. The solutions were filtered through filters with a pore size of 0.45 µm.

The 10 µL portions of standards and tablet samples solutions were spotted on the plates.

The developed chromatograms were air dried and visualized under UV light at 254 nm. Then, densitometric measurements were made with wavelengths determined prior to analysis.

Regression curves were plotted. For the analyzed compounds linearity was proven; for rupatadine in the range of 0.5–3 µg (Fig. 5) and for fexofenadine in the range of 5–60 µg (Fig. 6).

Determination of content in medical products

- Rupafin – 10 mg tablets (Rupatadine fumarate)
- Telfast 120 – 120 mg film-coated tablets (Fexofenadine hydrochloride)

The sample solutions of rupatadine (0.15 mg/mL) and fexofenadine (1 mg/mL) were prepared from commercially available Rupafin 10 mg tablets and Telfast 120 mg film coated tablets, respectively. The corresponding standard solutions for both examined substances were also prepared at the same concentrations.

The isolation of components from tablets was conducted by means of methanol. Samples were shaken for 14 min in an ultrasonic bath, then, they were shaken for 30 min in a mechanical shaker.

Table 2. Statistical assessment of the results concerning the determination of the tested compounds in pharmaceutical products.

<table>
<thead>
<tr>
<th>Tested compound</th>
<th>Method</th>
<th>Number of samples</th>
<th>Arithmetic mean of all measurements X [mg]</th>
<th>Standard deviation S [mg]</th>
<th>Confidence interval X ± ΔX PU = 95%</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rupafin tablets 10 mg</td>
<td>Densitometry</td>
<td>10</td>
<td>9.950 mg</td>
<td>0.04</td>
<td>9.950 ± 0.057 mg</td>
<td>0.41</td>
</tr>
<tr>
<td>(Rupatadine fumarate)</td>
<td>HPLC</td>
<td>10</td>
<td>9.910 mg</td>
<td>0.13</td>
<td>9.910 ± 0.079 mg</td>
<td>1.38</td>
</tr>
<tr>
<td>Telfast 120 coated tablets 120 mg</td>
<td>Densitometry</td>
<td>7</td>
<td>120.65 mg</td>
<td>0.73</td>
<td>120.65 ± 0.79 mg</td>
<td>0.60</td>
</tr>
<tr>
<td>(Fexofenadine)</td>
<td>HPLC</td>
<td>7</td>
<td>120.46 mg</td>
<td>0.70</td>
<td>120.46 ± 0.75 mg</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Figure 5. UV spectrum of fexofenadine measured directly from the TLC plate
Solutions were filtered through filters with a 0.45 µm pore size. The 10 µL portions of standard and sample solutions were transferred on silica gel F$_{254}$ HPTLC plates. After development with the mobile phase appropriate for each compound, the chromatograms were dried and the densitometric analysis was performed at determined wavelengths.

The results and statistical analysis are shown in Table 2.

In order to compare developed methods for the determination of examined substances in medicinal products, comparative analysis of rupatadine and fexofenadine was performed by HPLC methods described in the literature (7, 11). Fexofenadine was determined on a Zorbax C-8 (5 µm, 4.6 mm × 150 mm) column with UV detector at 210 nm. The mobile phase consisted of buffer acetate – acetonitrile – methanol in the ratio 60 : 20 : 20, v/v/v, adjusted to pH 3.7 (7). Rupatadine was determined on a column Lichrosphere C-8 (5 µm, 4.6 mm × 250 mm) with UV detector at 226 nm. The following mobile phase was used: methanol – buffer solution of potassium dihydrogen phosphate – acetonitrile (50:20:30, v/v/v) (11).

The obtained results are presented in Table 2.

RESULTS

Several different mobile phases and different types of chromatographic plates were investigated. For the qualitative analysis of both examined substances seven chromatographic systems were selected. Rf values and detection limits are summarized in Table 1; systems are described in section about identification.

Obtained results show that systems II, III and VII are the best to confirm the identity of rupatadine.

Figure 6. Densitogram of fexofenadine at 210 nm

Figure 7. Regression curve (dependence of chromatogram peak area (y) from the amount of rupatadine fumarate (x)).
Identification and determination of rupatadine and fexofenadine by... 1473
dine fumarate. System IV and optionally V or VI can be used to establish the purity of the compound. For best results, system IV and optionally III or VII are used to confirm the identity of fexofenadine. Other systems (I, II, V and VI) may be used to assess purity of the compound. RF values and broadening/blurr of chromatographic spots were taken into consideration when selecting system for quantitative analysis.

The following systems were used for quantitative analysis. System VII: Merck silica gel F254 HPTLC plates and mobile phase: acetonitrile – water – 25% ammonia (90 : 10 : 1, v/v/v) was selected for the determination of rupatadine (RF value of ca. 0.32).

System IV: Merck Silica gel RP 18 F254S HPTLC plates and mobile phase: acetonitrile – methanol – acetate buffer at pH 5.5 (3:2:5, v/v/v) proved to be the best for the quantification of fexofenadine (RF value of ca. 0.36).

Automatic sample applications were used to ensure the highest precision of densitometric measurements.

Conformity of chromatograms of examined substances isolated from Rupafin 10 mg tablets and Telfast 120 mg coated tablets and standard substances was demonstrated for all analyzed compounds.

Linearity was shown for both analyzed compounds in the range of 0.5-3.0 mg for and 5-60 mg for rupatadine and fexofenadine, respectively.

Coefficients of determination R² were 0.999 and 0.9985 for rupatadine and fexofenadine, respectively. Equations of curves are shown in Figures 5, 6. The limits of quantification were 0.3 µg and 5 µg for rupatadine and fexofenadine, respectively. The limits of detections were 0.1 µg and 2 µg for rupatadine and fexofenadine, respectively.

Simultaneously, in order to compare developed methods for the determination of examined substances in medicinal products, comparative analysis of discussed compounds was performed by HPLC methods described in the literature. The results are given in Table 2.

The statistical data obtained for developed densitometric methods and for comparative methods are similar.

CONCLUSIONS

Developed chromatographic system: Merck silica gel F254 HPTLC plates and mobile phase: acetonitrile – water – 25% ammonia at (90 : 10 : 1, v/v/v) ensures optimal identification conditions for rupatadine fumarate in Rupafin tablets 100 mg.

Developed chromatographic system: Merck silica gel F254 HPTLC plates and mobile phase: acetonitrile – methanol – acetate buffer at pH 5.5 (3:2:5, v/v/v) ensures optimal identification conditions for fexofenadine in Telfast tablets 120 mg.

Developed systems were used in densitometric determination of rupatadine fumarate and fexofenadine. Statistical data obtained for the developed assay methods indicate their sufficient precision and accuracy.

Not only are developed densitometric methods comparable to HPLC in terms of accuracy and precision, but also are cheaper and quicker to conduct. Hence, they can be routinely used to determine rupatadine fumarate and fexofenadine in medical products.

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2. Charakterystyka Produktu Leczniczego Telfast 120, 120 mg tabletki powlekane firmy Sanofi Aventis.

Figure 8. Regression curve (dependence of chromatogram peak area (y) from the amount of fexofenadine hydrochloride (x).

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