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DETERMINATION OF ACTIVE SUBSTANCES IN ANTIALLERGIC AND ANTIPHLOGISTIC MULTICOMPONENT PREPARATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract: The aim of the present study was the elaboration of an HPLC method enabling the identification and determination of the content of selected compounds occurring in multicomponent preparations applied in allergic and non–allergic diseases of upper respiratory tracts. These compounds include: buzepide methyl iodide, clocinizine dihydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine sulfate and dexbrompheniramine maleate. The elaborated HPLC method shows that a good separation of the mentioned compounds is feasible. The regression analysis has demonstrated linearity of the method in concentration range suitable for the intended experiments. The determination of the compounds in pharmaceutical preparations and the statistical evaluation of the results indicate that both the selectivity and precision of the method are good.

Keywords: phenylpropanolamine hydrochloride, clocinizine dihydrochloride, dexbrompheniramine maleate, buzepide methyl iodide, pseudoephedrine sulfate, HPLC method.

Diseases of the upper respiratory tracts, particularly of the nasal section, belong to the most frequently occurring illnesses. In view of the variety of infection reasons (bacterial infections, viremia and also allergic reactions), which could occur simultaneously, multicomponent medicines have found application. The mutually supplementary actions of antihistaminic sympaticomimetics cause the following: reduce the edema of nasal tract, contract the swollen nasal muscous tissues enabling unconstrained respiration, reduce the nasal eluate, sneezing, itching of the nose and throat, and dacryorrhea and burning in the eyes. To the active substances used in the production of multicomponent preparations, combining the above properties, belong, among others, the following compounds:

- phenylpropanolamine hydrochloride and pseudoephedrine from the sympaticomimetic amine group,
- clocinizine dichydrochloride and dexbrompheniramine maleate demonstrating the antihistaminic activity and
- buzepide methyl iodide from the parasympatholytic group.

The molecular structures, total and molecular weights are presented in Figure 1.

The preparations from the multicomponent medicament group, studied in this work, are given in Table 1.

Analytical studies of the mentioned preparations is a complicated task because of the presence of several components of significantly diversified quantities. There are some HPLC methods, which are used for the determination of other similarly acting compounds (1–7), however, mainly in the single form (3–7).

The aim of the present studies was the elaboration of a universal HPLC method enabling identification and determination of the content of the following selected compounds: buzepide methyl iodide, clocinizine dihydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine sulfate and dexbrompheniramine maleate, which may appear in various chemical combinations in pharmaceutical preparations.

EXPERIMENTAL

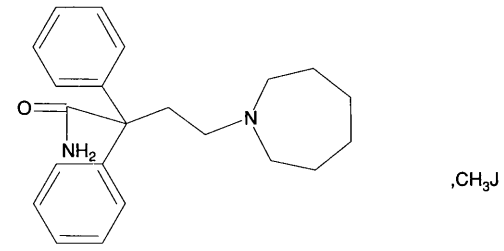
Materials

The following standard substances were used: clocinizine dihydrochloride, phenylpropanolamine hydrochloride, buzepide methyl iodide, dexbrompheniramine maleate and pseudoephedrine sulfate. The preparations are presented in Table 1.

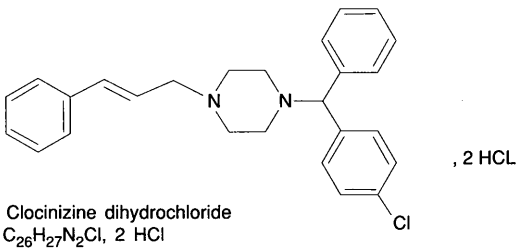
Chemicals and apparatus

The reagents used in this study were of high purity, suitable for HPLC.

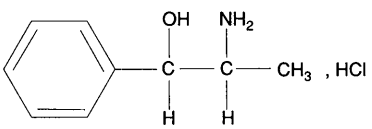
A liquid chromatograph, controlled by a computer (from Shimadzu), with a diode detector SPD–M10ATVP and a UV–VIS SPD–10AVP spectrometer with an LC–10ATVP pump and a DGU–14A degasser, a controller SCL–10AVP



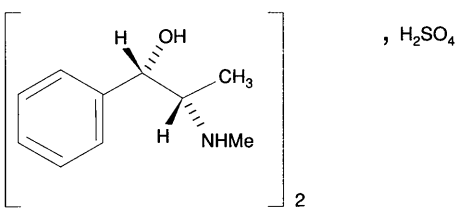
Buzepide methyl iodide
$C_{22}H_{28}N_2O$, CH_3J
M.W. 478.46



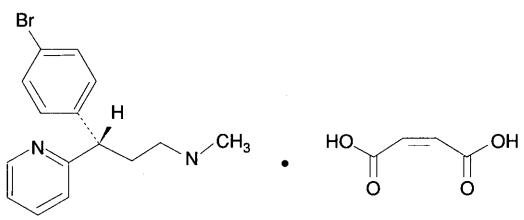
Clocinizine dihydrochloride
$C_{26}H_{27}N_2Cl$, 2 HCl
M.W. 475.89



Phenylpropanolamine hydrochloride
$C_9H_{14}ClNO$
M.W. 187.67



Pseudoephedrine sulfate
$(C_{10}H_{15}NO)_2$ H_2SO_4
M.W. 428.54



Dexbrompheniramine maleate
$C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$
M.W. 435.32

Figure 1. Molecular structures, total and molecular weights of the studied compounds.

and an autosampler SIL–10ADVP were used throughout.

HPLC experiments

As a first step, a search was carried out for an optimal system, which could enable identification and ensure good separation of the studied substances. Experiments were performed with the use of a diode detector of wavelengths ranging between 200 and 370 nm. To fix a wavelength rendering possible the determination of particular compounds, the course of chromatograms was analysed in that UV range and the spectra were drawn for each substance. A number of chromatographic systems, employing various columns and mobile phases, has been tested. To this end, a mixture of standards in methanol was prepared containing 0.2 mg in 1 ml of each: buzepide methyl iodide, clocinizine dihydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine sulfate and dexbrompheniramine maleate. A 10 µl portion of the solutions was injected into the column and the obtained chromatograms were analysed. For further studies the following system has been selected: a Phenomenex Spherisorb 5SCX 5 µm, 250x4.6 mm, column, a mobile phase containing a mixture of methanol and 0.03 $(NH_4)_2HPO_4$, adjusted to pH 6.0 with 85% orthophosphoric acid, (6:4). The flow rate of the mobile phase was 1.5 ml/min and the working temperature of the column was 40°C. The detection was performed at wavelengths of 220 and 254 nm with the use of the UV–VIS detector.

The chromatograms obtained under these conditions for the solution of the mixture of standards are shown in Figure 2. Values of the retention times for the studied substances are given in Table 2. In view of the great difference in the detection of the particular substances at the wavelength of 254 nm, for further experiments the 220 nm wavelength was chosen.

Determination of buzepide methyl iodide, clocinizine dihydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine sulfate and dexbrompheniramine maleate in pharmaceutical preparations

1. Construction of regression curves.

The elaborated HPLC system consisted of: a Phenomenex Spherisorb 5 SCX 5 µm, 250x4.6 mm column, a mobile phase – methanol : 0.03 M $(NH_4)_2HPO_4$, adjusted to pH 6.0 with 85% orthophosphoric acid, (6:4). The flow rate of the mobile phase was 1.5 ml/min. The working temperature of the column was 40°C. The detection was performed at a wavelength of 220 nm and the sample volume was 10 µl.

Table 1. Preparations used in this study

Preparation	Composition	Supplier
DENORAL tablets	buzepide methyl iodide, 1 mg clocinizine dihydrochloride, 5 mg phenylpropanolamine hydrochloride, 30 mg	Rhône – Poulenc Rorer
DISOPHROL coated tablets	pseudoephedrine sulfate, 120 mg dexbrompheniramine maleate, 6 mg	Schering – Plough
DRIXORAL coated tablets	pseudoephedrine sulfate, 120 mg dexbrompheniramine maleate, 6 mg	Schering – Plough
DISOPHROL syrup	pseudoephedrine sulfate, 6 mg/ml dexbrompheniramine maleate, 0.3 mg/ml	Schering – Plough

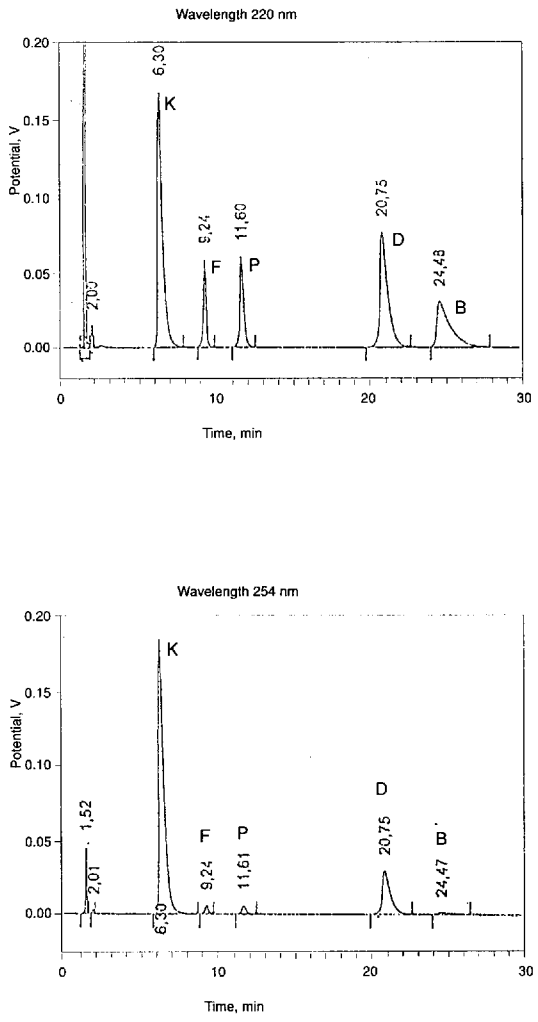


Figure 2. Chromatograms of a mixture of the five analysed substances observed at 220 and 254 nm.
B – buzepide methyl iodide, K – clocinizine dihydrochloride, F – phenylpropanolamine hydrochloride, P – pseudoephedrine sulfate, D – dexbrompheniramine maleate

The linearity of peak area, on the chromatograms in the concentration range of the analysed substances was examined as follows, for:

- buzepide methyl iodide 0.002–0.020 mg/ml (Figure 3);
- clocinizine dihydrochloride 0.0099–0.099 mg/ml (Figure 4);
- phenylpropanolamine hydrochloride 0.050–0.497 mg/ml (Figure 5);
- pseudoephedrine sulfate 0.100–0.899 mg/ml (Figure 6);
- dexbrompheniramine maleate 0.002–0.080 mg/ml (Figure 7).

The determined detection limits for the particular substances are presented in Table 2.

The determination of active substances in the preparations was performed in the conditions given at the description of the calibration of regression curves.

2. Determination of the content of buzepide methyl iodide, clocinizine dihydrochloride and phenylpropanolamine in the tablet preparation Denoral.

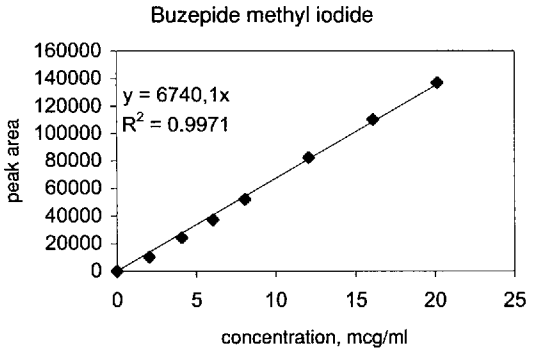


Figure 3. Dependence of the peak area (y) on the concentration of buzepide methyl iodide (x)

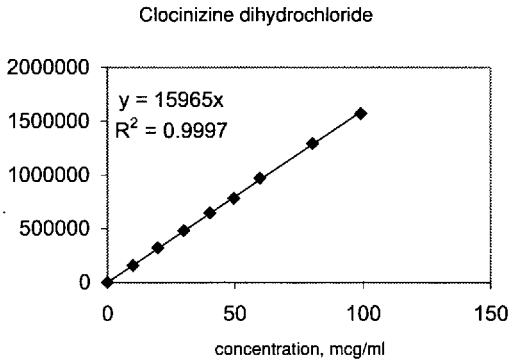


Figure 4. Dependence of the peak area (y) on the concentration of clocinizine dihydrochloride (x)

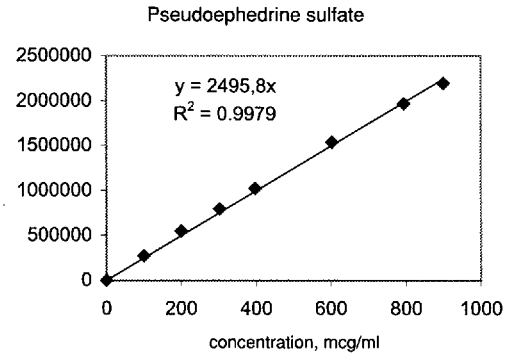


Figure 6. Dependence of the peak area (y) on the concentration of pseudoephedrine sulfate (x)

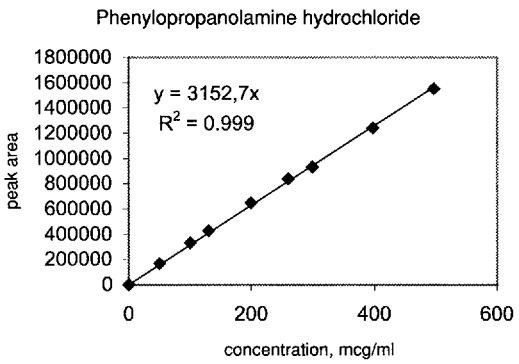


Figure 5. Dependence of the peak area (y) on the concentration of phenylpropanolamine hydrochloride (x)

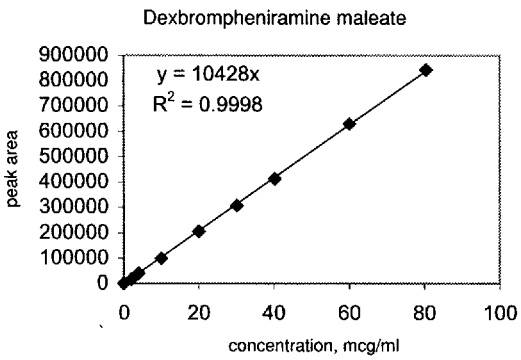


Figure 7. Dependence of the peak area (y) on the concentration of dexbrompheniramine maleate (x)

For their quantitative determination a standard solution and extract of the tablet, all in methanol, were prepared: 0.01 mg/ml of buzepide methyl iodide, 0.05 mg/ml of clocinizine dihydrochloride and 0.3 mg/ml of phenylpropanolamine hydrochloride. A 10 µl portion of the prepared solutions was injected into the column. The obtained results and their statistical evaluation are given in Table 3.

3. Determination of the content of pseudoephedrine and dexbrompheniramine in the preparations: Disophrol – coated tablets and Drixoral – coated tablets.

For the quantitative analysis, a standard solution and extract of the tablet, all in methanol, of concentration 0.6 mg/ml of pseudoephedrine sulfate and 0.03 mg/ml of dexbrompheniramine were prepared. A 10 µl portion of the prepared solutions was introduced into the chromatographic column. The results obtained and the statistical evaluation of the method are gathered in Table 4.

4. Determination of the content of pseudoephedrine sulfate and dexbrompheniramine in the preparation Disophrol – syrup.

For quantitative analysis, a standard solution and syrup solutions of concentration 0.6 mg/ml of pseudoephedrine sulfate and 0.03 mg/ml of dexbrompheniramine maleate in a methanol : water (1:1) mixture were prepared. A 10 µl portion of the prepared solutions was introduced into the chromatographic column. The results obtained and the statistical evaluation of the method are presented in Table 4.

RESULTS AND DISCUSSION

The objective of this study was the elaboration of an HPLC system which could allow to identify and determine five selected compounds applied in multicomponent preparations of antiallergic and antiphlogistic activity. The studied preparations

Table 2. Retention times and detection limits of the analysed substances.

Substance determined	Retention times, min	Detection limits ng/ml (injection 10 µl)
Buzepide methyl iodide	24.48	1018.0
Clocinizine dihydrochloride	6.30	79.20
Phenylpropanolamine hydrochloride	9.24	149.99
Pseudoephedrine sulfate	11.60	499.80
Dexbrompheniramine maleate	20.75	201.6

Table 3. Results and statistical evaluation of the determinations of the pharmacologically active compounds in Denoral tablets.

Substance determined	Declared content, mg	Average concentration, mg	Number of results n	Standard deviation S	95% confidence limit	Coefficient of variation, %
Buzepide methyl iodide	1	0.986	6	0.0139	0.986 ± 0.015	1.41
Clocinizine dihydrochloride	5	5.01	6	0.0694	5.010 ± 0.073	1.38
Phenylpropanolamine hydrochloride	30	29.44	6	0.4047	29.44 ± 0.425	1.37

Table 4. Results and statistical evaluation of the determinations of the pharmacologically active compounds in Disophrol coated tablets, Disophrol syrup and Drixoral coated tablets.

Preparation	Determined substance	Declared content tablets in mg, syrup in mg/ml	Average concentration, mg	Number of results n	Standard deviation S	95% confidence limit	Coefficient of variation
DISOPHROL coated tablets	Pseudoephedrine sulfate	120	119.80	6	1.0678	119.80 ± 1.121	0.89
	Dexbrompheniramine maleate	60	59.81	6	0.6141	59.81 ± 0.641	1.03
DISOPHROL syrup	Pseudoephedrine sulfate	6	6.17	6	0.0462	6.17 ± 0.048	0.75
	Dexbrompheniramine maleate	0.3	0.31	6	0.0032	0.31 ± 0.003	1.05
DRIXORAL coated tablets	Pseudoephedrine sulfate	120	121.61	6	0.7241	121.61 ± 0.760	0.59
	Dexbrompheniramine maleate	60	60.31	6	0.4182	60.31 ± 0.441	0.69

Table 5. Statistical evaluation of repeatability of injections of the standard solutions.

Substance determined	Average peak area	Number of results n	Standard deviation S	Coefficient of variation %
Buzepide methyl iodide	60722	6	571.95	0.94
Clocinizine dihydrochloride	723990.17	6	4856.79	0.67
Phenylpropanolamine hydrochloride	912739.5	6	6025.12	0.66
Pseudoephedrine sulfate	1563235.8	6	6420.03	0.41
Dexbrompheniramine maleate	295697.17	6	1481.38	0,50

included: Denoral and Disophrol tablets of prolonged action, Disophrol syrup and Drixoral tablets of prolonged action. At the first stage of this research, the possibility of simultaneous determination of these substances in various chromatographic systems was checked. To determine an optimal wavelength, chromatograms were recorded by means of the diode detector and spectra were drawn for each compound. Then, the determination was performed by employing the UV–VIS detector at two wavelengths: 220 and 254 nm. Because of the big differences observed in the detection of the particular substances at 254 nm (Figure 2), for further experiments the 220 wavelength has been selected. The studied system consisted of the Phenomenex Spherisorb 5 SCX 5 μm, 250x4.6 mm column and the mobile phase: methanol : 0.03 M $(NH_4)_2HPO_4$, adjusted to pH 6.0 with 85% orthophosphoric acid, (6:4). The flow rate of the mobile phase was 1.5 ml/min; the working temperature of the column was 40°C; detection was carried out at 220 nm; the sample volume was 10 μl. The above system has allowed a very good separation of all the five substances. The retention times for the particular compounds are given in Table 2.

Linearity of the method for each investigated compound was verified. Based on the regression analysis, a linear concentration range was established, which is as follows:

- buzepide methyl iodide from 0.002 to 0.020 mg/ml (Figure 3);
- clocinizine dihydrochloride from 0.0099 to 0.099 mg/ml (Figure 4);
- phenylpropanolamine hydrochloride from 0.050 to 0.497 mg/ml (Figure 5);
- pseudoephedrine sulfate from 0.100 to 0.899 mg/ml (Figure 6);
- dexbrompheniramine maleate from 0.002 to 0.080 mg/ml (Figure 7).

For each regression curve, the correlation coefficient, R^2, was determined, which is given in Figures 3–7. The method is demonstrating linearity in the required concentration range and the correlation coefficient, R^2, is reaching satisfactory values. By determining the detection limit for each compound, the sensitivity of the method has been estimated (Table 2). To obtain a more thorough estimation of the precision of the method, a series of injections of the standard solutions, prepared for the respective preparations, into the chromatographic system was carried out and the obtained results were statistically evaluated (Table 5).

Based on the obtained results, it was found that the elaborated HPLC system enables the identification and quantitative determination of each of the five studied substances, i.e. buzepide methyl iodide, clocinizine dihydrochloride, phenylpropanolamine hydrochloride, pseudoephedrine sulfate and dexbrompheniramine maleate. It is worth to notice that the method allows to determine simultaneously all five studied compounds.

In pharmaceutical preparations, applied in allergic and non–allergic diseases of the nasal part of the upper respiration tracts, the above substances may appear in any combinations. The method is characterized by good selectivity, precision and sensitivity. The linearity dependence of the peak area on the concentration of the studied substances was found in adequately big ranges, exeeding their content in curative preparations. This fact provides a high security of the accuracy of determinations of the content of the above compounds. The fact is worth to be mentioned that the accuracy and precision of the determinations is maintained even in preparations where big differences in concentration between the individual substances occur. Taking into account the quantity of the simultaneously determined compounds, it may be said that the proposed method is characterized by a short time of analysis (30 min), which is its additional advantage.

The elaborated method can be used in the analysis of pharmaceutical preparations containing the studied substances.

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