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## Validation of quantitative determination methods of diphenhydramine tablets by high performance liquid chromatography

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**Key words:** High Performance Liquid Chromatography (HPLC), Diphenhydramine, Validation Studies, Assay.

**Aim.** In order to quantify diphenhydramine tablets the methods based on reverse phase high-performance liquid chromatography with UV-spectrometry detection at 254 nm (HPLC-UV) have been used.

**Methods and results.** The mobile phase used a mixture of a phosphate buffer: acetonitrile (80:20). The proposed method is applied to the two dosage forms of diphenhydramine – tablets to 0.1 and 0.05, produced by various Ukrainian pharmaceutical companies. The main characteristics are validated by Pharmacopoeia of Ukraine.

**Conclusion.** According to the experimental data, the technique can be correctly reproduced, and is suitable for use in pharmaceutical and forensic analytic laboratories.

### Валідація методики кількісного визначення димедролу в таблетках методом високоефективної рідинної хроматографії

С. Л. Загородній, С. О. Васюк

З метою опрацювання та валідації за вимогами Державної Фармакопеї України зручної, економічної, високочутливої методики кількісного визначення димедролу у лікарських формах виконали високоефективну рідинну хроматографію. Протягом дослідження використали фармакопейний стандартний зразок димедролу, а також 2 зразки таблеток 0,1 г і 0,05 г, що вироблені різними українськими фармацевтичними підприємствами. Як рухома фазу використали суміш фосфатний буфер : ацетонітрil (80:20). Детекція – УФ-спектрометрична за довжини хвилі 254 нм. Визначили основні валідаційні характеристики за Державною Фармакопеею України. Згідно з експериментальними даними, методика може бути коректно відтворена та придатна для використання в лабораторіях Державної інспекції з контролю якості лікарських засобів, криміналістичних лабораторіях, а також відділах технічного контролю хіміко-фармацевтичних підприємств.

**Ключові слова:** високоефективна рідинна хроматографія, димедрол, валідація, кількісне визначення.

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### Валидация методики количественного определения димедрола в таблетках методом высокоэффективной жидкостной хроматографии

С. Л. Загородній, С. А. Васюк

С целью проработки и валидации в соответствии с требованиями Государственной Фармакопеи Украины удобной, экономичной, высокочувствительной методики количественного определения димедрола в лекарственных формах провели высокоэффективную жидкостную хроматографию. В ходе исследования использовали фармакопейных образец димедрола, а также 2 образца таблеток 0,1 г и 0,05 г, произведенных разными украинскими фармацевтическими предприятиями. В качестве подвижной фазы использована смесь фосфатный буфер: ацетонитрил (80:20). Детекция – УФ-спектрометрическая при длине волны 254 нм. Определены основные валидационные характеристики по Государственной Фармакопее Украины. В соответствии с экспериментальными данными, методика может быть корректно воспроизведена и пригодна для использования в лабораториях Государственной инспекции по контролю качества лекарственных средств, криминалистических лабораториях, а также отделах технического контроля химико-фармацевтических предприятий.

**Ключевые слова:** высокоэффективная жидкостная хроматография, димедрол, валидация, количественное определение.

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Diphenhydramine has a strong antihistamine and local anaesthetic activity, antispasmodic effect on the body and is one of the most popular members of antihistamines, H1-receptor blockers. In addition, diphenhydramine has a significant effect on the central nervous system, causing sedative and antiemetic effects [1]. Therapeutic activity of diphenhydramine and its low price ensures its popularity among manufacturers, pharmaceuticals and patients. In addition, severe effects on the central nervous system led to widespread misuse of diphenhydramine in non-medical purposes for potential use as narcotic drugs and psychotropic substances [2]. Thus ensuring quality control of dosage forms containing diphenhydramine, remains a pressing issue for modern pharmaceutical analysis [1, 3]. In addition,

for forensic chemical analysis it is required to find simple and reliable methods of quantitative analysis of diphenhydramine in dosage forms and other matrices. Efficiency analysis of drugs has significantly increased with the use of physical and chemical methods. It has long been the practice firmly entrenched chemical analysis by high performance liquid chromatography (HPLC), characterized by high sensitivity, specificity, simplicity and speed of analysis.

This method is recommended by Ukrainian Pharmacopoeia [4] and the European Pharmacopoeia [1].

Known methods of diphenhydramine analysis include gas chromatography – the lack of which is a long time analysis [6] – and capillary electrophoresis [7] and atomic emission spectroscopy

[8]. There are also methods of quantitative determination of diphenhydramine based on titrimetry [9], the method that despite the simplicity and accessibility is not characterized by high sensitivity. We also know several techniques of spectrometric determination of diphenhydramine. Some of them are based on measuring the optical density of the solution after the extraction of the reaction product of diphenhydramine Eriochrome Black T, methyl orange and bromocresol purple organic solvents [10], others methods are based on measuring the optical density of the solution of bromocresol green surplus after removal of the reaction product of diphenhydramine by extraction with organic solvent [11]. Such techniques require prior extraction of the active substance.

The literature describes numerous methods of quantitative determination of diphenhydramine, based on the use of high performance liquid chromatography, but it has not been validated by the Ukraine Pharmaceutical requirements [12-15].

### Purpose

Purpose of this paper is to study and validate the requirements for Ukrainian pharmacopoeia to find convenient, economical, highly sensitive methods of quantitative determination of diphenhydramine in dosage forms by high performance liquid chromatography.

### Materials and methods

#### Objects of research applied reagents and equipment

For this study, a sample Diphenhydramineum pharmacopoeia standard was used (SE «Ukrainian Research Center Pharmacopoeial quality of drugs» series 2) and medicines - pills «Diphenhydramine» 0.1 g (JSC «Kyiv Vitamin Factory», Ukraine) tablets «Diphenhydramine -Darnytsya «0.05 g (FF «Darnitsa», Ukraine), a series of 10 712 and EN31013 respectively. Solvents and reagents grade purity.

Analytical equipment: chromatograph Thermo Spectra System 2000, electronic scales ABT-120-5DM, measuring vessel class A.

#### General method of quantitative determination of diphenhydramine hydrochloride

Aliquot portion (0.0002 g) of diphenhydramine solution is used in the mobile phase and placed in a volumetric flask 10.00 mL, to bring the mobile phase to the mark and mix. Spend chromatography following conditions: column – reversed phase Hypersil Gold C18, Thermo Scientific TM, 4×150 mm, particle size sorbent – 5 mm, mobile phase – a mixture of phosphate buffer: acetonitrile in the ratio of 80:20, the flow rate – 1 ml/min, detector – UV spectrophotometric wavelength 254 nm, semi-injector volume of samples is 20 ml.

#### Preparation of phosphate buffer

In 1 liter of distilled water dissolve 3.0 g of potassium hydroxide, 12.0 g of 82% ortho-phosphoric acid and 3.0 g of diethylamine. The solution of said composition has a pH equal to 3. In the case of deviation from the specified pH transmitting correction, add ortho-phosphoric acid or potassium hydroxide.

#### Preparation of solutions for the mobile phase

Phosphate buffer should be mixed with acetonitrile in the ratio of 80:20, thoroughly mixed and filtered through a membrane filter with a pore size less than 20 microns.

#### Quantification determination of diphenhydramine in tablets

The exact sample of thoroughly pounded tablet weight (0.005 g aliquots diphenhydramine) should be dissolved in 3 ml of mobile phase in a glass of 25 ml and filtered in a volumetric flask 25.00 ml which also carry on filter, washed with an additional 2 ml of mobile phase two, the resulting solution should be adjusted to the mark with the same solvent and mixed. 1.00 ml of this solution is transferred to a volumetric flask 10.00 ml and analyzed by the general procedure. Calculation of the content of the active substance is carried out according to the typical formula.

### Results and discussion

HPLC method is widely used in chemical analysis for quantitative studies of drugs of different pharmacological groups and chemical properties. Firstly, this is due to the wide versatility method to no significant changes in terms of analysis to explore the different molecular structure of matter. Secondly, the relatively high selectivity of chromatographic performance is known in various substances.

The selected research method can be performed on equipment of broad spectrum. UV spectrometry detector combined with column allows you to explore a wide range of drugs. Also, this method does not use expensive and popular solvents - acetonitrile and phosphate buffer, and does not require long complex sample preparation. Therefore we can say that the chosen method best meets the intended purpose of our work.

At the preparatory stage of the study it was found that diphenhydramine is the main center in the molecule, so it should be well chromatographed on reverse phase column with a rather acidic mobile phase, which is the chosen mixture.

As a result chromatographic study, chromatograms containing one right diphenhydramine peak with retention time (t) 5.82 min, were obtained; this peak was absent in the chromatography blank sample (fig. 1).

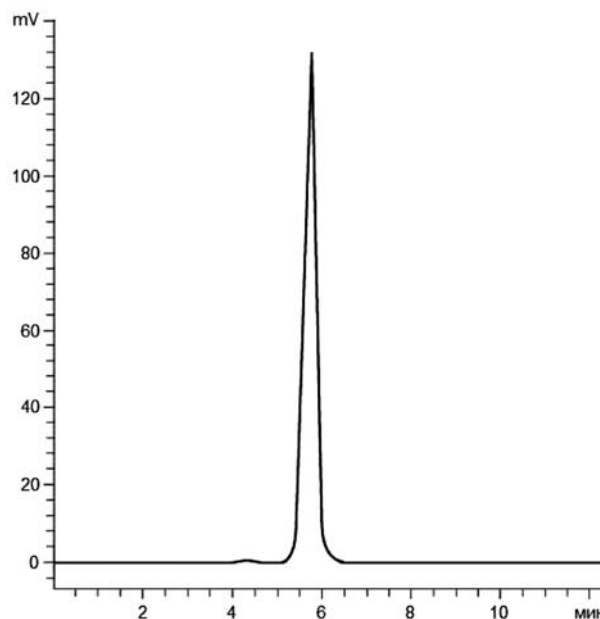


Fig. 1. Diphenhydramine chromatogram obtained for the selected method.



The basic chromatographic parameters obtained by chromatogram were calculated, such as retention volume (V), the symmetry factor (As), number of theoretical plates (N), the signal / noise (S / N), the maximum allowable relative standard deviation (RSDmax) and the relative standard deviation (RSD%). The value calculated indicators show the high efficiency of the selected chromatographic system (table 1).

Table 1

#### Main parameters of the selected chromatographic system efficiency

| t, min | V, ml | A <sub>s</sub> | N    | S/N | RSD <sub>max</sub> | RSD <sub>%</sub> |
|--------|-------|----------------|------|-----|--------------------|------------------|
| 5.82   | 5.82  | 1,07           | 1165 | 385 | 1.57               | 0.93             |

#### Validation of Analytical Procedures

According to the requirements of SPU validation procedure was carried out for the developed technique. Key validation specifications such as linearity, precision and accuracy were determined by a standardized procedure validation standard method [14].

*The linear dependence.* Linearity was determined within concentrations corresponding to 80–120% of nominal content of tablets.

Key figures linear relationship is shown in table 2, which shows that the technique confirm linearity over the entire range of concentrations mentioned above.

Table 2

#### Main parameters of linear dependence

| Size  | Value       | Criteria                            | Conclusion |
|---|-------------|-------------------------------------|------------|
| Tablets «Diphenhydramine» 0.1 g (JSC «Kyiv Vitamin Factory», Ukraine) |             |                                     |            |
| a±(S <sub>a</sub> )   | -56.6±(46)  | a≤Δa=t(95%; 3)·S <sub>a</sub> =108  | met        |
| S <sub>0</sub> /b   | 0.015       | ≤Δ <sub>As</sub> (%)/t(95%; 3)=1.02 | met        |
| r   | 0.9994      | ≥0.9979                             | met        |
| Tablets «Diphenhydramine-Darnitsya» 0.05 g (FF «Darnitsa», Ukraine)   |             |                                     |            |
| a±(S <sub>a</sub> )   | 63.6±(45,6) | a≤Δa=t(95%; 3)·S <sub>a</sub> =107  | met        |
| S <sub>0</sub> /b   | 0.015       | ≤Δ <sub>As</sub> (%)/t(95%;3)=1.02  | met        |
| r   | 0.9994      | ≥0.9979                             | met        |

*Precision methods* defined for each dosage form was determined at convergence. To do this, in each case parallel measurements were performed nine times (three sample three repetitions) and the results confirmed the expected validated characteristics (table 3). It was found that in all cases the one-sided confidence interval Δh does not exceed the maximum uncertainty analysis, so this method is accurate as at the level of convergence.

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Table 3

#### Determination of the convergence results of quantitative determination of drugs studied (n = 9, p = 0.95)

| Contents  | Metrological characteristics |                      |        |                                     |
|---|------------------------------|----------------------|--------|-------------------------------------|
|   | $\bar{X}$                    | S <sub>0</sub>       | RSD, % | $\Delta_{x,r} \leq \Delta_{As(\%)}$ |
| Tablets «Diphenhydramine» 0.1 g (JSC «Kyiv Vitamin Factory», Ukraine) |                              |                      |        |                                     |
| 0.1 g   | 0.1001                       | 1.4·10 <sup>-3</sup> | 1.40   | 2.6<3.20                            |
| Tablets «Diphenhydramine-Darnitsya» 0.05 g (FF «Darnitsa», Ukraine)   |                              |                      |        |                                     |
| 0.05 g  | 0.0501                       | 5.4·10 <sup>-4</sup> | 1.08   | 2.0<3.20                            |

*Correctness* in both cases was determined by supplementation. For this solution to the minimum sample three drugs were added in three different proportions of the sample solution standard pharmacopoeial diphenhydramine. Thus, three solutions of three concentrations of diphenhydramine were given. Next, the resulting solution was chromatographed by the method described above. The results can be considered accurate definitions, as systematic error is not statistically different from zero, that is the true value of the quantity does not exceed the specified confidence interval (table 4).

Table 4

#### Determination of the correctness of methods using the method supplements

| $\bar{Z}$   | RSD, % | Δ <sub>z</sub> | $ \bar{Z}-100  \leq \Delta_x / \sqrt{n}$ | Conclusion |
|---|--------|----------------|--|------------|
| Tablets «Diphenhydramine» 0.1 g (JSC «Kyiv Vitamin Factory», Ukraine) |        |                |  |            |
| 100.21  | 2.19   | 4.06           | 0.20<1.35                                | met        |
| Tablets «Diphenhydramine-Darnitsya» 0.05 g (FF «Darnitsa», Ukraine)   |        |                |  |            |
| 98.89   | 3.11   | 5.78           | 1.10<1.92                                | met        |

#### Conclusions

1. Studies of chromatography conditions of diphenhydramine were performed by HPLC. Accurate, economical and rapid chromatographic method of assay of diphenhydramine was chosen using mobile phase as a mixture of phosphate buffer: acetonitrile in the ratio of 80:20.

2. The calculated basic chromatographic parameters of diphenhydramine chromatogram were obtained with the selected method. They demonstrate the suitability of the chromatographic system according to the requirements of SPU quantitative analysis of diphenhydramine chosen method.

3. It had been proven that the method of quantification of processed diphenhydramine applied to two studied dosage forms for such characteristics as linearity, precision, accuracy is valid and can be used in pharmaceutical and forensic analytical laboratories.

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