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DEVELOPMENT OF NEW CRITERIA TO IDENTIFICATION AND QUANTITATIVE DETERMINATION OF THE COMPONENTS OF MULTICOMPONENT SYSTEMS BY CHROMATOGRAPHIC METHODS

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Key words: HPLC; chromatography; identification; quantitative determination; multicomponent systems

Currently a great number of drugs, which are complex multicomponent mixtures of natural and synthetic substances, are registered at the pharmaceutical market of Ukraine. While identifying multicomponent drugs by high performance liquid chromatography (HPLC) all components of mixtures are usually analyzed separately by their retention times; for each of the components the corresponding reference standard (RS) is used. To control the quality of multicomponent drugs we offer to use the ratio of peak areas of the substances to be analyzed obtained from the chromatogram of multicomponent mixtures with further normalization of these ratios. The ratios are suggested to be calculated by one of the drug components – a supporting agent. Testing of the method suggested has been carried out using the drug Ortofen-Zdorovye forte, coated tablets. Such dyes as Azorubine and Ponceau 4R is in the composition of the drug coating. To analyze these dyes the specific analytical wavelength – 500 nm has been chosen; Azorubine has been chosen as a supporting agent. Application of the given approach to determination of the dyes in the composition of the drug allows to complete the requirements of normative documents on the drug with the value of the ratio of the dyes peaks, and it is an additional guarantee of the drug quality. This new approach to identification and quantitative determination of the components of multicomponent systems by chromatographic methods allows to tighten regulations over the quality of drugs and fix the quantitative composition of the drug in rather narrow range. The application of the approach developed for the drug analysis allows also to simplify the pharmaceutical analysis significantly and make it cheaper.

Introduction of the Good Manufacturing Practice to Ukrainian enterprises has led to the severization of requirements to the quality of the products manufactured.

In these conditions of increasing requirements to the quality of manufactured drugs the need arises to have new criteria of quality, which would allow to guarantee efficiency and safety of the products at lower cost [5].

The last versions of monographs of the European Pharmacopoeia and the State Pharmacopoeia of Ukraine contain a sufficiently great number of the quality parameters allowing to control not only the qualitative composition but also the quantitative content of a drug. However, these parameters are effective only in quality control of monocomponent drugs or drugs, which contain some known active substances and excipients [7, 10].

At present while identifying multicomponent drugs by high performance liquid chromatography (HPLC) the approach when all components of mixtures are analyzed separately by their retention times is used. To identify each of the components the corresponding reference standard (RS) is used, as a rule. Such approach is costly as for time and finance since RSs are expensive part of analysis. Besides, by no means always the RS of some component of a multicomponent mixture is commercially avaliable (currently there are no pharmacopoeial reference standards of dyes) [3].

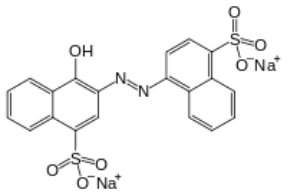
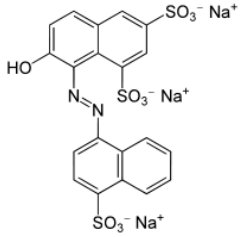
According to the Pharmacopoeia requirements, quantitative determination in multicomponent systems can be performed by both selective methods (HPLC), and non-selective methods (spectrophotometry, titration). In the first case the main problem is the absence of avaliable RSs of all components determined, in the second case calculation of the sum of all components with reference to one substance randomly chosen open broadest possibilities for adulteration of drugs.

The solution of the problems mentioned is application of only chromatographic methods for quantitative determination and identification of substances in multicomponent systems. However, the abovementioned problems with using RS do not allow to apply currently this approach.

To implement the approach described we offer to use the ratio of peak areas of the substances to be analyzed obtained from the chromatogram of multicomponent mixtures with further normalization of these ratios. The ratios are calculated by one of the drug components – a supporting agent. The basic criteria of the supporting agent selection are a high concentration of the

Table

Characteristics of Azorubine and Ponceau 4R

Name	Structural formula	Absorption maximum
Azorubine		510 nm [6]
Ponceau 4R		508 nm [6]

component in the mixture and a good separation of this substance with other substances of the mixture.

To determine the concentration of the supporting agent in the mixture the solution of the substance, which concentration is directly proportional to the label claim of the supporting agent in the drug, as well as the solution of the drug studied were prepared. The chromatographic procedure was carried out in conditions of the validated method with subsequent calculation of the concentration of the supporting agent. Then dispersion and the guaranteed permissible content were calculated for the concentration obtained for the tested substance [2]. Based on the calculation results obtained the maximum deviation for the ratio of the peaks analyzed was determined.

Therefore, for validated methods used in pharmaceutical analysis, identification and quantitative determination of the components (possessing linearity, correctness, precision and reproducibility) the possibility of introduction of the content normalization of the drug components appears since the ratio of peak areas corresponding to the components on chromatograms at constancy of the concentration ratio of these components in the drug should change in a rather narrow range [3].

Difference between the extinction coefficients for various components does not affect the results of analysis since to determine the drug quality the ratio of peak areas is used but not their absolute value, i.e. this difference is automatically taken into account when determining the nominal ratio from chromatograms of the standardized test solution [1].

Experimental Part

To test the method described the drug Ortofen-Zdorovye forte, coated tablets, has been chosen. The main problem in routine analysis of this drug is variability of the coating tint.

For identification of dyes such analytical methods as thin layer chromatography (TLC) [9], spectrophotometry and high performance liquid chromatography are used. Of the analytical methods mentioned it is HPLC that gives the most massive opportunities in unique identification of organic dyes in mixtures as the most selective method of analysis [8].

Such dyes as Azorubine (0.104 mg/tbl) and Ponceau 4R (0.066 mg/tbl) is in the composition of the drug coating.

The information concerning these dyes is given in the Table.

To analyze these dyes the specific analytical wavelength – 500 nm was chosen. It allowed to get only the peaks of the dyes indicated on the chromatograms since only intensely coloured substances absorbed in the range of 500 nm. The solution of Azorubine (supporting dye) was prepared in the amount of 1.04 mg/100 ml. The analysis was carried out under the following conditions:

- the column with the size of 0.25 m ×4.0 mm, filled with the sorbent and stationary phase containing octadecyl bonded silica gel for chromatography with the particle size of 5 μm (XTerra RP18, Waters Corp.), for which the requirements of the system suitability test should be met;
- mobile phase A: buffer solution with pH=3 degassed by any convenient way (place 0.68 g of potassium dihydrogen phosphate, 0.87 g of potassium hydrogen phosphate, 1.7 g of tetrabutylammonium iodide into a 1000.0 ml volumetric flask, dissolve in 900 ml of water, dilute to the volume with water, mix and adjust pH to (3.0+0.2) with phosphoric acid);
- mobile phase B: acetonitrile for chromatography degassed by any convenient way;
- the following gradient programme is used:

Time, min	MP A, % v/v	MP B, % v/v
0	80	20
0→20	80→35	20→65
20→21	35→80	65→20
21→25	80	20

- the rate of the mobile phase is 1.0 ml/min;
- detection wavelength is 500 nm.

The test solution (10 tablets of the drug per 100 ml of the solution) and the solution of Azorubine were chromatographed. The chromatogram of the test solution of the drug is given in Fig. 1. The chromatogram of Azorubine solution is given in Fig. 2. The content of Azorubine in the drug was calculated. The average content of 5 parallel measurements was 100.5%. Dispersion by 5 measurements was 0.874. RSD = 0.87 (at the maximum RSD=1.19 for 5 measurements [3]).

The ratio of the peak area of Ponceau 4R to the peak area of Azorubine was 0.60; the relative retention time of Ponceau 4R was 0.56 in relation to the peak of Azorubine.

Calculation of the guaranteed permissible content of Azorubine is as follows.

The real average result of quantitative determination of azorubine in the drug was A = 100.5% of the

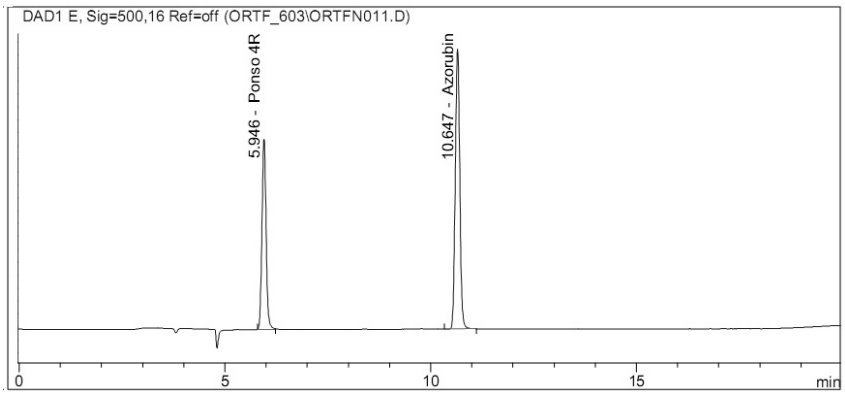


Fig. 1. The chromatogram of the tested solution.

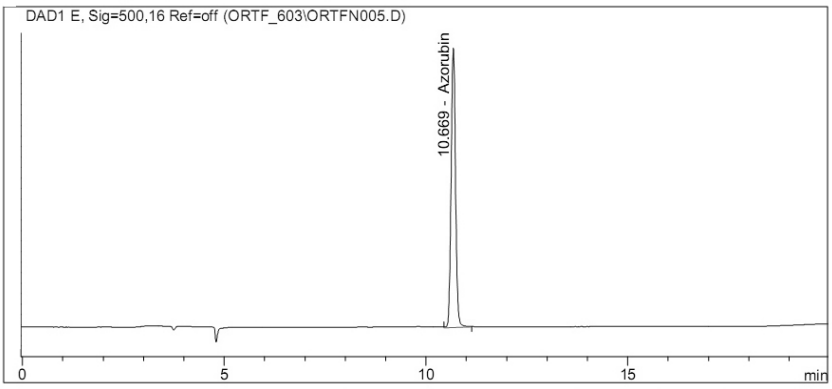


Fig. 2. The chromatogram of Azorubine solution.

label claim (5 measurements). This value A is in the range of $a_{min} \leq A \leq a_{max}$. The values A_{max} and A_{min} were calculated by formulas 1.1 and 1.2 [2]:

$$A_{min} = a_{min} + \frac{U(P_1) \cdot s}{\sqrt{m}}, \quad (1.1)$$

$$A_{max} = a_{max} - \frac{U(P_1) \cdot s}{\sqrt{m}}. \quad (1.2)$$

For quantitative determination $a_{min} = 95\%$, $a_{max} = 105\%$. Then at P_1=95% we obtain:

$$A_{min} = 95 + \frac{1.65 \cdot 0.874}{\sqrt{5}} = 95.64\%,$$

$$A_{mix} = 105 - \frac{1.65 \cdot 0.874}{\sqrt{5}} = 104.36\%.$$

Application of the given approach to the dyes in the composition of the drug allows to complete the requirements of normative documents on the drug with the value of the ratio of the dyes peaks since with the fixed ratio of dyes in the drug its final colour will be also in a rather narrow range, and it is an additional guarantee of the drug quality.

CONCLUSIONS

The authors of the given article have suggested a new approach to identification and quantitative determination of the components of multicomponent systems, in particular drugs, which allows together with the unique identification to control the quantitative content of all components as well. The method allows to tighten regulations over the quality of drugs and fix the ratio of all components of the drug irrespective of whether the component is determined quantitatively or only its presence is identified.

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РОЗРОБКА НОВИХ КРИТЕРІЇВ ДО ІДЕНТИФІКАЦІЇ ТА КІЛЬКІСНОГО ВИЗНАЧЕННЯ КОМПОНЕНТІВ БАГАТОКОМПОНЕНТНИХ СИСТЕМ ХРОМАТОГРАФІЧНИМИ МЕТОДАМИ

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Ключові слова: *ВЕРХ; хроматографія; ідентифікація; кількісне визначення; багатокомпонентні системи*

Останнім часом на фармацевтичному ринку України реєструється значна кількість лікарських препаратів, які являють собою складні багатокомпонентні суміші природних і синтетичних речовин. При ідентифікації багатокомпонентних лікарських препаратів методом високоефективної рідинної хроматографії усі компоненти сумішей звичайно аналізуються окремо за часом утримання, для кожного з компонентів використовується стандартний зразок. Для контролю якості багатокомпонентних лікарських препаратів запропоновано використовувати відношення площ піків аналізованих речовин, отриманих із хроматограм багатокомпонентних сумішей, з наступним нормуванням цих відношень; відношення пропонується розраховувати по одному з компонентів препарату – «опорному». Апробація запропонованого методу проведена на препараті «Ортофен – Здоров'я форте», таблетки, вкриті оболонкою. До складу оболонки даного препарату входять барвники Азорубін і Понсо 4R. Для аналізу вказаних барвників була вибрана специфічна аналітична довжина хвилі – 500 нм, в якості «опорного» компонента використовували Азорубін. Такий підхід до визначення барвників, що входять до складу препарату, дозволяє доповнити вимоги нормативної документації на препарат значенням відношення піків барвників, що додатково гарантує якість препаратів. Запропонований новий підхід до ідентифікації і кількісного визначення компонентів багатокомпонентних систем хроматографічними методами дозволяє значно підвищити вимоги до якості лікарських засобів і зафіксувати кількісний склад препарату в досить вузьких межах. Також використання розробленого підходу до аналізу лікарських препаратів дзволяє значно спростити і здешевити фармацевтичний аналіз.

РАЗРАБОТКА НОВЫХ КРИТЕРИЕВ К ИДЕНТИФИКАЦИИ И КОЛИЧЕСТВЕННОМУ ОПРЕДЕЛЕНИЮ КОМПОНЕНТОВ МНОГОКОМПОНЕНТНЫХ СИСТЕМ ХРОМАТОГРАФИЧЕСКИМИ МЕТОДАМИ

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Ключевые слова: *ВЭЖХ; хроматография; идентификация; количественное определение; многокомпонентные системы*

В последнее время на фармацевтическом рынке Украины регистрируется значительное количество лекарственных препаратов, представляющими собой сложные многокомпонентные смеси природных и синтетических веществ. При идентификации многокомпонентных лекарственных препаратов методом высокоэффективной жидкостной хроматографии все компоненты смесей обычно анализируются отдельно по временам удерживания, для каждого из них используется стандартный образец. Для контроля качества многокомпонентных лекарственных препаратов предлагается использовать отношение площадей пиков анализируемых веществ, получаемых из хроматограмм многокомпонентных смесей, с последующим нормированием этих отношений; отношения предлагается рассчитывать по одному из компонентов препарата – «опорному». Апробация предложенного метода проведена на препарате «Ортофен-Здоровье форте», таблетки, покрытые оболочкой. В состав оболочки данного препарата входят красители Азорубин и Понсо 4R. Для анализа данных красителей была выбрана специфичная аналитическая длина волны – 500 нм, в качестве «опорного» компонента использовали Азорубин. Применение данного подхода к определению красителей, входящих в состав препарата, позволяет дополнить требования нормативной документации на препарат значением отношения пиков красителей, что дополнительно гарантирует качество препарата. Предложенный новый подход к идентификации и количественному определению компонентов многокомпонентных систем хроматографическими методами позволяет ужесточить требования к качеству лекарственных средств и зафиксировать количественный состав препарата в достаточно узких пределах. Также применение разработанного подхода к анализу лекарственных препаратов позволяет значительно упростить и удешевить фармацевтический анализ.