MULTISENSORY COLORIMETRIC ANALYSIS OF DRUGS DYDROGESTERONE, TROXERUTIN AND ADEMETIONINE USING BARCODES

O.V. Monogarova1, A.A. Chaplenko1,2, K.V. Oskolok1

1 Lomonosov Moscow State University
1-3, Leninskie gory, Moscow, Russia, 119991
2 I.M. Sechenov First Moscow State Medical University (Sechenov University) 
2-4 Bolshaya Pirogovskaya str., Moscow, Russia, 119435

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The aim of this study is to develop a universal, rapid and affordable method for the identification of dydrogesterone, troxerutin, and ademetionine in drugs by multisensor digital colorimetry using a unique two-dimensional code. The developed approach can be applied to rapid detection of counterfeit drugs at the preliminary stage of the analysis (before using more expensive specialized equipment).

Materials and methods. To implement the proposed approach, the substances of dydrogesterone ("Abbott Biologicals B.V.", Netherlands), troxerutin (JSC “Interfarma”, Prague, Czech Republic) and ademetionine (LLC “Farmamed”, Moscow, Russia), troxerutin capsules 300 mg (LLC “Pranafarm”, Samara, Russia), were used. A multisensor colorimetry method has been implemented using the following set of 8 sensors (C1–C8): an intact solution – a 96% (v/v) aqueous ethanol solution – C1; 1 mM alcoholic solution of anthraquinone green (CAS#4403-90-1) – C2; a 0.2% aqueous solution of 3-methylbenzothiazoline–3-hydrazone (CAS#1128-67-2) – C3; a 0.2% methyl orange aqueous solution (CAS#547-58-0) – C4; a 1 mM alcoholic solution of sulforhodamine B (CAS#3520-42-1) – C5; a 0.2% methyl orange aqueous solution (CAS#547-58-0) – C6; a 1 mM alcoholic solution of allura red AC (CAS#25956-17-6) – C7; a 1 mM aqueous solution of iron (III) chloride – C8. Transparent flat-bottomed polypropylene plates with 96 cells, with a cell volume of 350 µl (Thermo Fischer Scientific, USA, cat. No. 430341) were used as a base for the chip. For obtaining raster images, an Epson Perfection 1670 office flatbed scanner (CCD-matrix) with a removable cover was used. The obtained digital images of the cells were processed using the ImageJ software (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) with a 24-bit RGB color model (8 bits per channel).

Results. The adequacy of the developed approach was confirmed by the analysis of the above-listed drugs. It has been shown that the results obtained have no statistically significant differences from the values determined by the spectrophotometric method.

Conclusion. The possibility of using multisensor digital colorimetry for pharmaceutical analysis has been shown. The developed methods for the identification of the active substances can serve as a good supplement to more expensive traditional methods.

Keywords: dydrogesterone; troxerutin; ademetionine; digital multisensor colorimetry; barcode

Abbreviations: RGB – red, green, blue; MBTH – 3-methylbenzothiazolinone hydrazone; PCA – Principal Component Analysis; PC1 – Principal Component 1.

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Цель. Разработка универсального экспрессного и доступного способа определения дидрогестерона, троксерутина и адеметионина в лекарственных препаратах методом мультисенсорной цифровой цветометрии с использованием уникального двумерного кода. Разработанный подход может быть применен для быстрого выявления фальсификатов лекарственных средств на предварительном этапе анализа (до использования более дорогого специализированного оборудования).

Материалы и методы. Для реализации предложенного подхода использованы субстанции дидрогестерона («Эбботт Билоджикалл Б.В.», Нидерланды), троксерутина (АО «Интерфарма», Прага, Чехия), адеметионина (ООО «Фармамед», Москва, Россия), капсулы троксерутина 300 мг (ООО «Пранафарм», Самара, Россия), лиофилизат для приготовления раствора для внутривенного и внутримышечного введения «Гептрал» (адеметионин) 400 мг («Эбботт Лабораторис», ГмБХ, Германия), таблетки «Дюфастон» (дидрогестерон) 10 мг («Эбботт Хелсек Продактс Б.В.»), Нидерланды). Метод мультисенсорной цветометрии реализован с использованием следующего набора из 8 сенсоров (C1–C8): интактный раствор – 96% (v/v) водный раствор этанола – C1; 1 мМ спиртовой раствор антрахинонового зеленого (CAS#4403-90-1) – C2; 0,2% водный раствор метилбензотиазолинон-гидразона (CAS#1128-67-2) – C3; 0,2% водный раствор метилбензотиазолинон-гидразона (CAS#547-58-0) – C4; 1 мМ спиртовой раствор сульфородамина B (CAS#3520-42-1) – C5; 1 мМ спиртовой раствор 1-гидроксипирена (CAS#6315-79-7) – C6; 1 мМ спиртовой раствор красного очаровательного AC (CAS#25956-17-6) – C7; 1 мМ водный раствор железа (III) хлорида – C8. В качестве основы для чипа использовали прозрачные планшеты из полипропилена с плоским дном на 96 ячеек, объем ячейки – 350 мкл (Термо Фишер Сциентифик, США, кат. № 430341). Для получения растровых изображений применяли офисный планшетный сканер Epson Perfection 1670 (CCD-матрица) со съемной крышкой. Полученные цифровые изображения ячеек обрабатывали в программе ImageJ (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) с использованием цветовой модели RGB 24 бит (8 бит на канал).

Результаты. Адекватность разработанного подхода подтверждена при анализе вышеперечисленных лекарственных препаратов. Показано, что полученные результаты не имеют статистически значимых отличий от величин, определённых спектрофотометрическим методом.

Заключение. Показана возможность использования мультисенсорной цифровой цветометрии для фармацевтического анализа. Разработанные способы определения действующих веществ могут служить хорошим дополнением к более дорогостоящим традиционным методам.

Ключевые слова: дидрогестерон; троксерутин; адеметионин; цифровая мультисенсорная цветометрия; штрих-код.

Список сокращений: RGB (КЦС) – красный, зеленый, синий; МБТГ – метилбензотиазолинон-гидразон; PC1 – главная компонента 1 / Principal Component 1; PCA – метод главных компонент / principal component analysis.

INTRODUCTION

For a preliminary rapid detection of counterfeits (even before using more expensive analytical equipment), it is advisable to practise simple, accessible and express methods. One of these methods is a digital colorimetry, based on the registration of electromagnetic radiation in the visible range of wavelengths by digital devices to get color raster images [1–6]. Digital colorimetry has become widespread in pharmaceutical analyses. In this area, the method is used to: analyze medicinal plants [7, 8]; assess the quality of collections, which include powders of medicinal plants [9–11]; determine the whiteness of powdered and tableted drugs [12]; identify the biologically active substances and drugs both by their own color and by the color of the products of color reactions used in pharmacopoeial tests [12]; identify the DOA and banned products [13, 14].

Digital colorimetry combines the availability of chemical test methods with a visual detection and a good performance of instrumental methods, primarily optical molecular spectroscopy. The extremely low cost of the analysis by this method is due to the possibility to measure the analytical signal using...
consumer digital optical devices not certified as measuring instruments [1, 5, 15, 16]. Despite the obvious advantages, the colorimetric method is not devoid of a number of limitations, the main one of which is its low selectivity [4, 17].

To increase the selectivity of the method, the use of molecular sensors was proposed [18]. It is advisable to use a cell of several chromogenic agents as sensors, in which a series of analytical reactions can be carried out simultaneously. The multisensor colorimetry method [19–29] is based on obtaining colored products of an analyte interaction with molecular sensors, getting information about their color characteristics, and then converting them into a discrete substance “barcode” that can be used for chemical analyses [17, 30].

A unique colorimetric two-dimensional code makes it possible to estimate both the nature and the content of active substances in the drugs at the minimum level of information noise [17, 30]. To form the “barcodes”, it is advisable to choose such sensors and color channels, the values of the lightness of which correlate with the content of the analyte. Lightness shall mean the color coordinate on one of the color channels in the RGB system (varies in the range from 0 to 255).

The drugs of three different pharmacological groups were selected as the test objects. Dydrogesterone is a synthetic progestogen that fully ensures the onset of the secretion phase in the endometrium in cases of endometriosis and dysmenorrhea. Troxerutin is a flavonoid, a phleboprotective drug that has venotonic, angioprotective, anti-inflammatory, anti-edema and antioxidant effects. Ademetionine is an antioxidant, hepatoprotective, detoxifying agent. The structural formulas of the active substances are shown in Fig. 1. The development of alternative methods for their identification, suitable for a preliminary screening analyses of drugs, is an important and urgent task of the pharmaceutical and analytical chemistry.

THE AIM of this study is to develop a universal method by multisensor digital colorimetric analysis of drugs of various pharmacological groups using dydrogesterone, troxerutin and ademetionine as examples. The developed complex of molecular sensors in combination with new approaches to the processing of analytical signals will make it possible to identify the above-mentioned active substances in the drugs.

MATERIALS AND METHODS

Study objects

To implement the proposed approach, the substances of dydrogesterone (“Abbott Biologicals B.V.”, Netherlands), troxerutin (JSC “Interfarma”, Prague, Czech Republic) and ademetionine (LLC “Farmamed”, Moscow, Russia), troxerutin capsules 300 mg (LLC “Puranfarm”, Samara, Russia), lyophilisate for an intravenous solution and an intramuscular administration of “Heptral®” (ademetionine) 400 mg (“Abbott Laboratories”, GMBH, Germany), tablets “Duphaston®” (dydrogesterone) 10 mg (“Abbott Healthcare Products B.V.”, Netherlands) were used.

Materials

For a quantitative analysis, a series of calibration solutions was prepared using the substances troxerutin and ademetionine (4.0-20.0 mg/ml) in increments of 4 mg/ml, dydrogesterone (1.0-3.0 mg/ml) in increments of 0.5 mg/ml. The concentration range had been selected in such a way that the content of the active substance in the real drug would be in the middle of the calibration curve.

The calibration solutions were analyzed by multisensor colorimetry using the following set of 8 sensors (C<sub>i</sub>–C<sub>j</sub>): an intact solution – a 96% (v/v) aqueous ethanol solution – C<sub>j</sub>; 1 mM alcohol solution of anthraquinone green (CAS#4403-90-1) – C<sub>j</sub>; a 0.2% aqueous solution of 3-methylbenzothiazolinone hydrazone (MBTH) (CAS#1128-67-2) – C<sub>j</sub>; a 1 mM alcoholic orange aqueous solution (CAS#547-58-0) – C<sub>j</sub>; a 1 mM alcoholic solution of sulforhodamine B (CAS#3520-42-1) – C<sub>j</sub>; a 1 mM alcoholic solution of 1-hydroxypyrrene (CAS#5315-79-7) – C<sub>j</sub>; 1 mM alcoholic solution of allura red AC (CAS#25956-17-6) – C<sub>j</sub>; a 1 mM aqueous solution of of iron (III) chloride – C<sub>j</sub>.

Equipment

Transparent flat-bottomed polypropylene plates with 96 wells [31–33], cell volume 350 µl (Thermo Fischer Scientific, USA, cat. No. 430341) were used as a base for the chip.

Using Biohit mLine dispensers (Sartorius, USA), 100 µl of alcohol solutions of substances, sensor solutions (C<sub>i</sub>–C<sub>j</sub>), and purified water were placed into the cells of the plate. The number of sensors was determined so that it would be possible to analyze the maximum number of samples on one plate (8 sensors by the number of rows of the plate).

For obtaining raster images, an Epson Perfection 1670 office flatbed scanner ( CCD-matrix) with a removable cover was used. The plate with the samples was scanned using the Epson Scan software in the Professional mode (600 dpi resolution, 24-bit color depth). “Color restoration”, “Unsharp mask filter” and “Descreening filter” options were disabled. To perform a digital colorimetric analysis using a 96-cell plate (Thermo Fischer Scientific, USA, cat. No. 1256604), a Teflon insert of 210×297×17 mm in size, was made with a center rectangular cut (128×86 mm) and was placed under the cover of an office A4 flatbed scanner. It made it possible to: expedite and formalize the procedure for placing the plate on the working glass table of the scanner; fix the coordinates and lighting conditions of the plate with an electroluminescent lamp built into the carriage; minimize the side stray illumination of the plate with substrates by external illumination sources; improve the accuracy of measuring results of plate raster images color channels lightness.

The difference in the color channels lightness between the analyte cell and the intact cell was used as an analytical signal. The obtained digital images of the cells were processed using the ImageJ software (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) using the RGB 24-bit color model (8 bits per channel), in each cell the central area was selected and 3 averaged values of lightness were obtained for it, one for each color channel of RGB. The choice of color channels was carried out empirically.
RESULTS AND DISCUSSION

Semi-quantitative colorimetric analysis of troxerutin, dydrogesterone and ademetionine

The obtained values of the lightness of RGB-channels were processed in the MS Excel spreadsheet editor, the optimal threshold values of the difference in the lightness of the channels for the analyzed solution and the intact cell were chosen. The values above them were conventionally designated as “1” and below them as “0” (Table 1) and colorimetric “barcodes” were created (Table 2). When choosing the optimal threshold value for the difference in lightness, the following requirements were met: (1) the code must be unique; (2) the difference in coding between adjacent concentrations should be minimal (1-2 values). To meet these requirements, it is advisable to set individual thresholds for each channel. This problem was solved using MS Excel (Add-in “Search for a solution”).

The presented one-dimensional “barcodes” can be clustered into a two-dimensional code (Table 3), which makes it possible both to estimate the nature and the content of the active substance in the drugs at the minimum level of the information noise. The interpretation of a two-dimensional code for the identification of the substances is possible both in visual and “instrumental” mode, for example, using a software “barcode” scanner on a smartphone after its preliminary setup. The latter mode is especially useful when processing large data sets to increase the reliability of the analysis results.

Thus, the technique of the semi-quantitative analysis of drugs can be reduced to comparing the code of the test solution with the corresponding code of a standard solution of the known concentration. Since the inaccuracy of the semi-quantitative analysis results is initially high, there is no need to use an inaccessible standard sample. It is just necessary to reproduce the described conditions of measuring the analytical signal and use a ready-made set of two-dimensional barcodes.

Colorimetric quantitative analysis of dydrogesterone, troxerutin and ademetionine drugs

For the quantitative analysis, it is advisable not to use all color channels and sensors, but only those the lightness values of which correlate with the analyte content. The coefficients of determination (r2) are calculated for all analytes, sensors and color channels, sensors and channels for which the value of r2 > 0.99 is a linearity criterion for the pharmaceutical analysis methods are identified. Thus, for the analysis of troxerutin, 4 color channels were selected (G4, G5, R7 and R8), for dydrogesterone – 5 channels (R2, G4, G6, B6 and R7), for ademetionine – 6 channels (R2, G2, R3, B3, G5 and G7).

To test the developed approach, a colorimetric analysis of the following drugs was carried out: tablets of dydrogesterone “Duphaston”® 10 mg, capsules of troxerutin 300 mg and hyophilate of ademetionine “Heptral”® 400 mg. In order to select the optimal method for the identification of active substances, a comparison of the metrological characteristics of the methods using all the proposed color channels and sensors, was carried out. The content of the active substance in the drugs was determined by the calibration curve method. The results of the active substances identification in the indicated drugs using the developed approach, are presented in Table 4.

For all variants of colorimetric techniques, the equality of the means was proved using the modified Student’s t-test for independent samples (P>0.95). The table shows that methods of the troxerutin identification using the R-channel of sensor 7, of ademetionine – the G-channel of sensor 2, and of dydrogesterone – the R-channel of sensor 2, have the best metrological characteristics. The presented data show that the results of the analysis of the drugs by method of multisensor digital colorimetry, accord well with the data declared by the manufacturer (obtained by high performance liquid chromatography and spectrophotometric method).

Using the technique of the principal component analysis for the assay of dydrogesterone, troxerutin and ademetionine drugs.

An approach in which the set of lightness values of color channels is considered as a kind of “colorimetric spectrum”, seems promising. In this case the data can be processed using chemometric algorithms, of which the principal component analysis (PCA) is used most often. In this case, it is possible, on the one hand, to select all useful information from all sensors on all channels at once, on the other hand, the level of the information noise can be reduced and the accuracy of the analysis results can be increased.

To test chemometric approaches, a series of calibration solutions of the troxerutin and ademetionine substances (4.0–20.0 mg/ml) in increments of 4 mg/ml, and dydrogesterone (1.0–3.0 mg/ml) with a step of 0.5 mg/ml were used. The values of the first principal component (PC1) were calculated by the formulas.

For dydrogesterone:

\[
\begin{align*}
PC1 &= -0.01 \Delta R_1 - 0.31 \Delta R_2 - 0.02 \Delta G_3 - 0.23 \Delta B_7 - \\
&- 0.01 \Delta R_1 - 0.01 \Delta G_5 - 0.35 \Delta B_7 - 0.01 \Delta R_1 - 0.21 \Delta G_7 - 0.01 \Delta R_1 + \\
&+ 0.01 \Delta R_7 - 0.44 \Delta G_6 - 0.40 \Delta B_3 - 0.01 \Delta R_1 - 0.09 \Delta G_5 - 0.22 \Delta B_7 - \\
&- 0.24 \Delta R_7 - 0.09 \Delta G_5 - 0.02 \Delta B_6 - 0.04 \Delta R_1 - 0.46 \Delta G_8 - 0.03 \Delta B_8 \\
\end{align*}
\]

For troxerutin:

\[
\begin{align*}
PC1 &= 0.02 \Delta R_5 + 0.10 \Delta G_1 + 0.05 \Delta B_6 + 0.38 \Delta R_7 + 0.01 \Delta G_3 + 0.31 \Delta B_3 + \\
&+ 0.17 \Delta R_3 + 0.48 \Delta G_7 + 0.21 \Delta B_8 + 0.13 \Delta G_5 + 0.14 \Delta B_3 + \\
&+ 0.23 \Delta R_7 + 0.16 \Delta G_7 + 0.38 \Delta B_8 + 0.17 \Delta R_3 + 0.18 \Delta G_3 + \\
&+ 0.23 \Delta R_3 + 0.01 \Delta G_3 + 0.02 \Delta B_6 + 0.27 \Delta R_3 - 0.02 \Delta G_8 + 0.02 \Delta B_8 \\
\end{align*}
\]
Figure 1 – Structural formulas of dydrogesterone (a), troxerutin (b), ademetionine (c)

Table 1 – Colorimetric codes corresponding to various concentrations of dydrogesterone, troxerutin and ademetionine

|                     | Dydrogesterone | Troxerutin | Ademetionine |
|---------------------|----------------|------------|--------------|
| c, mg/ml            | ΔR₂  | ΔG₄  | ΔG₆  | ΔB₆  | ΔR₈  | ΔG₈  | ΔR₉  | ΔG₉  | ΔG₁₀ |
| Threshold value of  |       |       |       |       |       |       |       |       |       |
| differences in     | 127  | 92   | 30   | 50   | 80   |       |       |       |       |
| lightness           |       |       |       |       |       |       |       |       |       |
| 1.0                 | 0    | 0    | 0    | 0    | 1    |       |       |       |       |
| 1.5                 | 1    | 0    | 0    | 0    | 1    |       |       |       |       |
| 2.0                 | 1    | 0    | 0    | 1    | 1    |       |       |       |       |
| 2.5                 | 1    | 1    | 0    | 1    | 1    |       |       |       |       |
| 3.0                 | 1    | 1    | 1    | 1    | 1    |       |       |       |       |
| Threshold value of  |       |       |       |       |       |       |       |       |       |
| differences in     | 125  | 91   | 82   | 92   |       |       |       |       |       |
| lightness           |       |       |       |       |       |       |       |       |       |
| 4 or less           | 0    | 0    | 0    | 0    | 0    |       |       |       |       |
| 8                   | 1    | 0    | 0    | 0    | 0    |       |       |       |       |
| 12                  | 1    | 1    | 0    | 0    | 0    |       |       |       |       |
| 16                  | 1    | 1    | 1    | 0    | 0    |       |       |       |       |
| 20                  | 1    | 1    | 1    | 1    | 1    |       |       |       |       |
| Threshold value of  |       |       |       |       |       |       |       |       |       |
| differences in     | 127  | 92   | 30   | 50   | 80   | 101   |       |       |       |
| lightness           |       |       |       |       |       |       |       |       |       |
| 4                   | 0    | 0    | 0    | 0    | 1    | 0     |       |       |       |
| 8                   | 0    | 1    | 1    | 0    | 1    | 0     |       |       |       |
| 12                  | 0    | 1    | 1    | 1    | 1    | 0     |       |       |       |
| 16                  | 0    | 1    | 1    | 1    | 1    | 1     |       |       |       |
| 20                  | 1    | 1    | 1    | 1    | 1    | 1     |       |       |       |
Table 2 – Scale of “barcodes” corresponding to various concentrations of dydrogesterone, troxerutin and ademetionine

| Active substance, c, mg/ml | Dydrogesterone “Barcode” | Troxerutin “Barcode” | Ademetionine “Barcode” |
|---------------------------|--------------------------|----------------------|------------------------|
| 1.0                       | 4                        | 4                    |                        |
| 1.5                       | 8                        | 8                    |                        |
| 2.0                       | 12                       | 12                   |                        |
| 2.5                       | 16                       | 16                   |                        |
| 3.0                       | 20                       | 20                   |                        |

Table 3 – Two-dimensional “barcodes” for simultaneous analysis dihydrosterone, troxerutin and ademetionine

| Active substance, c, mg/ml | C₁ | C₂ | C₃ | C₄ | C₅ | C₆ | C₇ | C₈ |
|---------------------------|----|----|----|----|----|----|----|----|
| Dydrogesterone (2.5 mg/ml)| R  | G  | B  | R  | G  | B  | R  | G  |
| 1.0                       |    |    |    |    |    |    |    |    |
| 1.5                       |    |    |    |    |    |    |    |    |
| 2.0                       |    |    |    |    |    |    |    |    |
| 2.5                       |    |    |    |    |    |    |    |    |
| 3.0                       |    |    |    |    |    |    |    |    |
| Troxerutin (16 mg/ml)     | R  | G  | B  | R  | G  | B  | R  | G  |
| 4                          |    |    |    |    |    |    |    |    |
| 8                          |    |    |    |    |    |    |    |    |
| 12                         |    |    |    |    |    |    |    |    |
| 16                         |    |    |    |    |    |    |    |    |
| 20                         |    |    |    |    |    |    |    |    |
| Ademetionine (8 mg/ml)    | R  | G  | B  | R  | G  | B  | R  | G  |
| 4                          |    |    |    |    |    |    |    |    |
| 8                          |    |    |    |    |    |    |    |    |
| 12                         |    |    |    |    |    |    |    |    |
| 16                         |    |    |    |    |    |    |    |    |
| 20                         |    |    |    |    |    |    |    |    |

Note: C₁–C₈ – sensors; the dark fill of the cell corresponds to the presence of a signal, the light one – to its absence
Table 4 – Results of active substances identification in medicinal products by multisensor digital colorimetry using various color channels and sensors

| Sensor and color channel | Active ingredient content, mg/unit | Spectrophotometry (n = 3, P = 0.95) | Digital colorimetry (n = 11, P = 0.95) | S² (for digital colorimetry) |
|-------------------------|------------------------------------|------------------------------------|----------------------------------------|-----------------------------|
| Dydrogesterone          |                                    |                                    |                                        |                             |
| R₁                      | 11.1 ± 1.2                         | 11.0 ± 0.8                         | 0.048                                  |
| G₄                      | 8.4 ± 1.0                          |                                    | 0.053                                  |
| G₅                      | 7.0 ± 0.8                          |                                    | 0.050                                  |
| B₅                      | 6.2 ± 0.6                          |                                    | 0.042                                  |
| R₇                      | 14.4 ± 1.7                         |                                    | 0.053                                  |
| Troxerutin              |                                    |                                    |                                        |                             |
| G₄                      | 294 ± 23                           |                                    | 0.036                                  |
| G₅                      | 284 ± 25                           |                                    | 0.040                                  |
| R₇                      | 290 ± 20                           |                                    | 0.036                                  |
| R₉                      | 291 ± 18                           |                                    | 0.028                                  |
| Ademetionine            |                                    |                                    |                                        |                             |
| R₁                      | 393 ± 42                           |                                    | 0.048                                  |
| G₁                      | 395 ± 19                           |                                    | 0.022                                  |
| R₃                      | 389 ± 27                           |                                    | 0.031                                  |
| B₃                      | 388 ± 28                           |                                    | 0.033                                  |
| G₇                      | 400 ± 35                           |                                    | 0.040                                  |
| G₉                      | 376 ± 24                           |                                    | 0.029                                  |

Table 5 – Results of multisensor colorimetric identification of active substances in drugs by the principal component analysis

| Active ingredient content, mg/unit | Spectrophotometry (n = 3, P = 0.95) | Digital colorimetry (n = 11, P = 0.95) | S² (for digital colorimetry) |
|-----------------------------------|-------------------------------------|----------------------------------------|-----------------------------|
| Dydrogesterone                    | 10.2 ± 0.1                          | 11.0 ± 0.8                             | 0.031                       |
| Troxerutin                         | 287 ± 2                             | 290 ± 7                                | 0.016                       |
| Ademetionine                       | 391 ± 4                             | 388 ± 9                                | 0.020                       |

Figure 2 – Dependence of the first main component vs concentration of dydrogesterone (a), troxerutin (b), ademetionine (c) in calibration solutions
It can be notified that there is a linear correlation between the value of the first main component (PC1) and the content of dydrogesterone, troxerutin and ademetionine in calibration solutions (Fig. 2), which can be used to determine the content of these active substances in the drugs. The results of the analyses of the drugs for the identification of the active substances in the drugs using the developed approach, are presented in Table 5. The results obtained, accord well with the data declared by the manufacturer. Tables 4 and 5 show that the use of the principal component analysis improves the reproducibility of the analysis results in comparison with the use of the calibration dependence for the selected sensor and color channel.

CONCLUSION
An efficient approach (potentially having a wide application) has been proposed for a screening analysis of drugs of various pharmacological groups by multisensor digital colorimetry after a preliminary sample preparation. The simultaneous use of several chemical sensors in a chip provides sufficient selectivity. Discretization of the multisensor signal makes it possible to generate a unique barcode suitable for the identification of the active substances in drugs. The developed methods for the identification of active substances can serve as a good supplement to more expensive traditional methods.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION
All authors have equally contributed to the research work.

REFERENCES
1. Apyari VV, Gorbunova MV, Isachenko AI, Dmitrienko SG, Zolotov YuA. The use of consumer color-registering devices in quantitative chemical analysis. Zhurnal analiticheskoi khimii. 2017;11(72):963–977. DOI: 10.7868/S0044450217110019. Russian.
2. Ivanov VM, Monogarova OV, Oskolov KV. Opportunities and prospects for the development of the colorimetric method in analytical chemistry. Zhurnal analiticheskoi khimii. 2015;10(70):1011–1025. DOI: 10.7868/S0044450215100114. Russian.
3. Monogarova OV, Oskolov KV, Apyari VV. Colorimetry in chemical analysis. Zhurnal analiticheskoi khimii. 2018;11(73):857–867. DOI: 10.1134/S0044450218110063. Russian.
4. Khimchenko S.V., Eksperiandova L.P. Colorimetry in instrumental and visual test analysis. Lambert Academic Publishing. 2014:220 p. Russian.
5. Shultz EV, Monogarova OV, Oskolov KV. Digital colorimetry: analytical potential and prospects of use. Vestnik Moskovskogo universiteta. Series 2: Chemistry. 2019;2(60):79–87. DOI: 10.3103/S002713141902007X. Russian.
6. Chernousova OV, Rudakov OB. Digital images in analytical chemistry for quantitative and qualitative analysis. Khimiya, fizika i mekhanika materialov. 2019;2(21):55–125. Russian.
7. Pogotskaya AA, Buzuk GN. The use of the scanner and software digital image processing for quantitative determination of alkaloids in the leaves of plume poppy. Vestnik farmatsii. 2009;4(46):32–38. Russian.
8. Ershik OA, Buzuk GN. The use of a scanner and computer software for digital image processing for the quantitative determination of phenolic compounds of rhizomes with roots of marsh cinquefoil. Vestnik farmatsii. 2008;4(42):6–12. Russian.
9. Ivankova MN, Buzuk GN. Colorimetric method to determine the composition of powders from medicinal plant materials. Vestnik farmatsii. 2010;4(50):22–28. Russian.
10. Vernigorova MN, Buzuk GN. Colorimetric method to determine the component composition of herbal powders of bur beggar-ticks (BIDENS TRIPARTITA L.). Vestnik farmatsii. 2013;4(62):28–33. Russian.
11. Buzuk GN, Kuzmicheva NA. Colorimetric and densitometric methods of analysis in the standardization of tablets “Ascorutin” and “Rutascorbin”. Vestnik farmatsii. 2011;3(53):12–18. Russian.
12. Rudakov LA, Vasilieva AP, Shvedov GI, Povlapovskaya EV. Digital technologies for determining the color and whiteness of drugs. Farmatevtsicheskii tekhnikologii i upakovka. 2012;2(215):38–40. Russian.
13. Choodum A, Daeid NN. Rapid and semi-quantitative presumptive tests for opioid drugs. Talanta. 2011;86:284–292. DOI: 10.1016/j.talanta.2011.09.015.
14. Choodum A, Parabun K, Daeid NN, Kanatharana P, Wongsiramaikul W. Real time quantitative colorimetric test
for methamphetamine detection using digital and mobile phone technology. Forensic Science International. 2014;123(5):8–13. DOI: 10.1016/j.forsciint.2013.11.018.

15. Oskolok KV, Shultz EV, Monogarova OV, Chaplenko AA. Optical molecular analysis using office flatbed photo scanner: new approaches and solutions. Talanta. 2018;178:377–383. DOI: 10.1016/j.talanta.2017.09.049.

16. Oskolok KV, Shultz EV, Monogarova OV, Chaplenko AA. Optical molecular analysis of pharmaceuticals using an office flatbed scanner: colorimetry and photometry. Voprosy biologicheskoi, meditsinskoi i farmatsevticheskoi khimii. 2017;8(20):22–27. Russian.

17. Monogarova OV, Chaplenko AA, Oskolok KV. Multisensory digital colorimetry to identify and determination of active substances in drugs. Sensors and Actuators, B: Chemical. 2019;(299). DOI: 10.1016/j.snb.2019.126909.

18. Ushakov EN, Alfimov MV, Gromov SP. Principles of design of optical molecular sensors and photoreceptors based on crown ethers. Uspekhi khimii. 2008;77(7):39–59. Russian.

19. Kangas MJ, Ernest A, Lukowicz RM, Mora AV, Quossi A, Perez M, Kyes N, Holmes AE. The identification of seven chemical warfare mimics using a colorimetric array. Sensors. 2018;18(291):1–8. DOI: 10.3390/s1812291.

20. Kangas MJ, Wilson KL, Burks LM, Atwater J, Lukowicz RM, Garver B, Mayer M, Havenridge S, Holmes AE. An improved comparison of chemometric analysis for the identification of acids and bases with colorimetric sensor arrays. International Journal of Chemistry. 2018;10(36):55. DOI: 10.5539/ijc.v10n2p36.

21. Kangas MJ, Burks RM, Atwater J, Lukowicz RM, Garver B, Holmes AE. Comparative chemometric analysis for classification of acids and bases via a colorimetric sensor array. Journal of Chemometrics. 2017; e2961. DOI: 10.1002/jcm.2961.

22. Zhang C, Bailey DP, Suslick KS. Colorimetric sensor arrays for the analysis of beers: A feasibility study // Journal of Agricultural and Food Chemistry. 2006;14(54):4925–4931. DOI: 10.1021/jf060110a.

23. Zhang C, Suslick KS. A colorimetric sensor array for organics in water. Journal of the American Chemical Society. 2005;33(127):11548–11549. DOI: 10.1021/ja052606z.

24. Palacios MA, Wang Z, Montes VA, Zryyanov GV, Anzenbacher Jr. Rational design of a minimal size sensor array for metal ion detection. Journal of the American Chemical Society. 2008;130(130):10307–10314. DOI: 10.1021/ja080237k.

25. Feng L, Musto CJ, Keibling JW, Lim SH, Zhong W, Suslick KS. Colorimetric sensor array for determination and identification of toxic industrial chemicals. Analytical Chemistry. 2010;82(22):9433–9440. DOI: 10.1021/ac100886.

26. Lin H, Suslick KS. A colorimetric sensor array for detection of triacetone triperoxide vapor. Journal of the American Chemical Society. 2010;132(15):15519–15521. DOI: 10.1021/ja107419t.

27. Carey JR, Suslick KS, Hulkeyer KL, Imlay JA, Imlay KRC, Ingison CK, Ponder JB, Sen A, Wittig AE. Rapid identification of bacteria with a disposable colorimetric sensing array. Journal of the American Chemical Society. 2011;133(17):7571–7576. DOI: 10.1021/ja201634d.

28. Suslick BA, Feng L, Suslick KS. Discrimination of complex mixtures by a colorimetric sensor array: coffee aromas. Analytical Chemistry. 2010;82(22):2067–2073. DOI: 10.1021/ac902823w.

29. Goodey A. Development of multianalyte sensor arrays composed of chemically derivatized polymeric microspheres localized in micromachined cavities. Journal of the American Chemical Society. 2001;123(2559–2570. DOI: 10.1021/ja003411.

30. Monogarova O.V., Chaplenko A.A., Oskolok K.V. Identification and determination of chloramphenicol in drugs by multisensor digital colorimetry. Vestnik Moskovskogo Universiteta. Series 2: Chemistry. 2020;1(61):3–10. DOI: 10.3103/S0027131420010071. Russian.

31. Johnke H. Detecting concentration of analytes with DETECHIP: a molecular sensing array. Journal of Sensor Technology. 2013;3(3):94–99. DOI: 10.4236/jst.2013.33001.

32. Smith A. Improved image analysis of DETECHIP® allows for increased specificity in drug discrimination. Journal of Forensic Research. 2012;8(3):161–164. DOI: 10.4172/2157-7145.1000161.

33. Okuom MO, Holmes AE. Developing a color-based molecular sensing device: DETECHIP®. Sensors & Transducers. 2014;12(183):30–33.

AUTHORS

Oksana V. Monogarova – Associate Professor, Candidate of Sciences (Chemistry), Lomonosov Moscow State University, Department of Chemistry, Analytical Chemistry Division. ORCID ID: 0000-0002-5790-1462. E-mail: o_monogarova@mail.ru

Alekandr A. Chaplenko – Associate Professor, Candidate of Sciences (Pharmacy), I.M. Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0003-1176-4658. E-mail: a.achaplenko@yandex.ru

Kirill V. Oskolok – Associate Professor, Candidate of Sciences (Chemistry), Lomonosov Moscow State University, Department of Chemistry, Analytical Chemistry Division. ORCID ID: 0000-0002-7785-4835. E-mail: k_oskolok@mail.ru