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DEVELOPMENT AND STANDARDIZATION OF TEST SYSTEMS BASED ON FILTER PAPER AND MODIFIED WITH VANILLIN REAGENT

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Реактиви та аналітичні інструменти для проведення експрес-аналізу повинні бути стандартизовані та приведені до вимог Державної Фармакопеї України. Відсутність можливості стандартизації газетного паперу істотно ускладнює використання лігнінової проби як методики експрес-аналізу компонентів екстемпоральних лікарських засобів, що містять в своєму складі первинну ароматичну аміногрупу. Вирішенням даної проблеми може стати розробка тест-систем на основі фільтрувального паперу, модифікованого фармакопейними реактивами.

Мета. *Мета дослідження – розробка та в провадження в практику внутрішньоаптечного контролю якості тест-системи на основі фільтрувального паперу, для проведення експрес-аналізу екстемпоральних лікарських засобів, що містять в своїй структурі первинну ароматичну аміногрупу в умовах аптек.*

Методи. *Метод фізичної іммобілізації; визначення фізичної стабільності тест-систем; економіко-статистичні методи (розрахунок вартості); валідація аналітичних методик; статистичні методи обробки експериментальних даних хімічного експерименту.*

Результати. *Для створення тест-системи був використаний фільтрувального паперу і фармакопейний розчин реактиву ваніліну, можливість застосування тест-системи на практиці досліджувалася за допомогою експрес-аналізу похідних амідів кислоти сульфанілової – сульфацетаміду та сульфатіазолу натрію. Доведено можливість застосування розробленої тест-системи для ідентифікації 5 % водних розчинів похідних амідів кислоти сульфанілової, встановлена межа виявлення та визначені інтервали ненадійності для методики експрес-аналізу з використанням тест-систем, які склали 5,0–9,0 мг/мл для сульфацетаміду натрію і 5,3–9,6 мг/мл для сульфатіазолу натрію. Тест-система стабільна протягом 5-х місяців зберігання. Ціна виготовлення 1 тест-системи в умовах аптеки становить 0,34 і 0,16 грн. для першої і наступних партій тест-систем відповідно.*

Висновки. *Запропонована тест-система є стабільною і доступною до використання в умовах аптек в якості аналітичного інструменту для проведення експрес-аналізу сполук похідних амідів кислоти сульфанілової*

Ключові слова: *екстемпоральні лікарські засоби, хімічні тест-системи, експрес-аналіз, лігнінова проба, сульфацетамід натрію, сульфатіазол натрію*

1. Introduction

Despite the widespread use of polymeric materials as matrices for the immobilization of reagents on the test kits, usage of cellulosic and filter paper bases for the manufacturing of test kits is promising in the medical field, in particular for the determination of glucose in the blood [1]. Test kits can also be used in the practice of healthcare facilities and in home conditions [1]. This matrix is safe and available for use, so it can be used for test kits manufacturing intended for *in vivo* testing, including humans [2]. Paper bases are used for creation not only of chemical test kits, but also biochemical and biological test tools, in particular [3], for detection of Escherichia coli in food. It is widely known about test kits usage for pollution monitoring, such as test kits for semi-quantitative analysis of arsenic ions in drinking water [4]. There are modern developments of a test kits that allow the detection of concomitant substances and impurities in complex organic compounds, in particular the detection of Mg^{2+} in latex of natural rubber [5]. Due to its properties, paper matrices can be modified correspondingly [6] for various analytical problems solving that are posed to test-kits. The use of testing methods and, in particular, test systems on a paper basis as part of the confirmatory approach of the Quality Control (QC) [7, 8] allows optimizing the cost of reagents and the working time of the pharmacist-analyst [9].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The usage of the lignin test as the rapid method for the identification of active pharmaceutical ingredients (APIs) containing the primary aromatic amino group (PAAG) in its structure (sulfanilamide preparations, derivatives of the para-aminobenzoic acid, etc.) has a number of problems in the modern legal and regulatory framework of Ukraine. The main problem is the impossibility of proper validation of the methodology and standardization of the analytical tools used for this analysis in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPU). [10]. The successful development of the manufacturing technology and implementation of the confirmatory approach of the QC of the APIs with the aid of simple chemical test kits modified with the Fe^{3+}, Cu^{2+}, Co^{2+} metal salts [7, 8] gives the opportunity to apply the accumulated knowledge to create a standardized chemical test kit suitable for using in the practice of rapid analysis of APIs which contain PAAG in their structure.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

There exist inventions of test kits for analysis of compounds with PAAG based on gelatine films modified with vanillin and *n*-dimethylbenzaldehyde pharmacopoeia reagents [11]. Analysis of these analytical tools application shows acceptable results, but the usage of test kits based on the gelatin films for QC of compounded preparations in pharmacies is not feasible because of the high cost of the test system and the complicated procedure for its manufacturing.

The paper [12] introduces the results of procaine hydrochloride identification through the filter paper (FP), modified with solutions of aromatic aldehydes. Applying of this matrix is more cost-effective because of its low cost and availability.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

The main disadvantage of the lignin test rapid method is the use of non-bleached newsprint paper or pieces of wood as analytical tool. These analytical tools are available, but they can't be standardized. The usage of test kits based on FP, modified with aromatic aldehyde will make it possible to minimize this disadvantage of lignin test implementation.

The test kit must be accessible for manufacturing and usage in pharmacies, stable and suitable for long-term use, what is important, consider the short shelf life of reagents aromatic aldehydes [10].

5. Formulation of goals (tasks) of Article

The aim of the work is development and implementation of test-kits based on filter paper for rapid analysis of compounded preparations that contain primary aromatic amino group in conditions of pharmacies.

To achieve this goal, the following tasks were solved:

– the test kit based on FP modified pharmacopoeia vanillin solution was developed;

– the approval of this test kit for identification of APIs was carried out;

– the test kit stability parameters were studied.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

For test kits manufacturing the following materials were used: *matrix* – FP «Ф» mark; reagents: vanillin, crystalline $C_8H_8O_3$ batch No. 10215215, China; sodium sulfacetamide, sodium sulfathiazole, ethanol 96 % batch No. 081013, Ukraine.

Original and model solutions of sodium sulfacetamide and sodium sulfathiazole were made by dissolving exact weights of substances in purified water S to the adjusted concentration. The solution of vanillin was prepared according to the requirements of SPU [8].

Test kits preparation. Test-systems were prepared through physical immobilization, according to the techniques [10, 13], which was developed for test systems modified with metal salts: FP`s plates 50*80 mm were immersed in a solution of reagent for 2 minutes. After that, the plates were dried and cut into test strips 5*50 mm. Test kits were placed in a sealed container for storage. The test kits have a white colour and characteristic odour. The test system was labelled: ***FP (v)***.

Rapid analysis. The solutions of the substances selected for testing the suitability of the test kit in a volume of 1 drop were applied to the surface of the test system using a glass rod. The appearance of the analytical effect was observed within 5–30 seconds. The analytical effect was compared with the colouring of the blank experiments and parallel researches-comparisons.

The blank experiments and parallel researches-comparisons were carried in two versions each: the blank experiment No. 1 (b.e. No. 1) was an experiment where *purified water S* was used instead of the solution test substance; the blank experiment No. 2 (b.e. No. 2) was an experiment where FP was used instead of test kit; the researches-comparison No. 1 (r.c. No. 1) was f classic version of the lignin test using non-standardized newsprint, carried out according to the method [13]; the researches-comparison No. 2 (r.c. No. 2) was a reaction of condensation with aromatic aldehydes, carried out in a microtube using pharmacopoeia reagents according with the method [13].

Derivatives of sulfanilic acid amides: sodium sulfacetamide and sodium sulfathiazole were selected to test the suitability of the developed test kit in concentrations widely used in the compounded preparations (50 mg/ml aqueous solution).

Suitability analysis. The results of the research are given in Table 1. Since the lignin test is not a specific method for identifying compounds with PAAG, the analytical effects were identical obtained for both substances.

Table 1

Analytical effects on the plane of the test kit

substance	***FP (v)***	the researches-comparisons		the blank experiments	
		r.c. No. 1	r.c. No. 2	b.e. No. 1	b.e. No. 2
sodium sulfacetamide	yellow colour	yellow colour	orange colour	no effect	no effect
sodium sulfathiazole	yellow colour	yellow colour	orange colour	no effect	no effect

The analytical effect on the surface of the test kit complies with effect obtained in the researche-comparison No. 1 (bright yellow colour). The analytical effect on the surface of newsprint (orange colour) differs from the test kit, which may be explained by the unspecified quality and quantity composition of newsprint. The colour on the plane of the test kit is clear, unlike newsprint, what makes the procedure of identification with this technique more comfortable and quick. In the blank experiments the absence of analytical effect was observed. The blank experiments No. 2 was carried out to determine the matrix influence on the analytical effect of the reaction.

In the main experiment, only the solution of the test substance was applied on the surface of the test kit, without the addition of a solution of sulphuric acid diluted. At the same time, the difference in the analytical effects between the main and the r.c. No. 1 was not observed, what indicates about the optimization of the list of necessary reagents with using of test kit for the identification of substances containing PAAG.

The reliability study. The model solutions of sodium sulfacetamide and sodium sulfathiazole at concentrations of 35.0, 42.5, 50, 57.5 and 65.0 mg/ml were prepared for study of reliability of reaction results reproducibility in the range of use 70–130 % of the initial concentration [14, 15]. The research was carried out according to the standardized procedure [14, 15] in conditions of 3 laboratories. The results of the research are presented in Table 2.

Table 2

Data of the identification reaction results reproducibility of the derivatives of sulfanilic acid amides with the help of the vanillin-modified test kit

Range of use, %	**70**	**85**	**100**	**115**	**130**
sodium sulfacetamide					
C, mg/ml	35,09	42,45	50,11	57,59	65,13
R, %	100	100	100	100	100
Δ C	30,04				
sodium sulfathiazole					
C, mg/ml	35,15	42,63	50,56	57,89	65,11
R, %	100	100	100	100	100
Δ C	29,96				

It was found that the use of a vanillin-modified test kit is characterized by 100 % assurance in the results of reaction reproducibility in a range corresponding to 5 % aqueous solution for the both derivatives of sulfanilic acid amides.

The study of unreliability intervals. For sensitivity determination of the developed test kit, the study of the detection limits of substances and the unreliability intervals of this identification technique (Table 3) was carried out. Based on experimental data the "effectiveness curve" was built (Fig. 1).

Table 3

The study of the intervals of unreliability of detection of the derivatives of sulfanilic acid amides with the help of the test kit

mg/ml	∞/P	4	5	6	7	8	9	10	11	$C_{0,05}$, *mg/ml*	$C_{0,95}$, *mg/ml*	ΔC, *mg/ml*	$\frac{\Delta C}{C_{0,05}}$
sodium sulfacetamide	$\overline{P_{C_k}}$	0	0,05	0,25	0,40	0,75	0,95	1,00	1,00	5,0	9,0	4,0	0,80
	$\hat{\alpha}_k$	1,00	0,95	0,75	0,60	0,25	0,05	0	0				
sodium sulfathiazole	$\overline{P_{C_k}}$	0	0	0,20	0,45	0,65	0,90	1,00	1,00	5,3	9,6	4,3	0,81
	$\hat{\alpha}_k$	1,00	1,00	0,80	0,55	0,35	0,10	0	0				

The intervals of unreliability of the identification of sodium sulfacetamide using the test kit of ***FP (v)*** is 5.0–9.0 mg/ml, which indicates about the acceptability of this technique for the identification sodium sulfacetamide starting at the concentration of 0.9 %; the unreliability intervals of the method of detecting sodium sulfathiazole is 5.3–9.6 mg/ml, respectively, the concentration of this substance, which can be identified with an acceptable reliability is 0.96 %.

Stability study. Considering that the vanillin reagent of SPU and the European Pharmacopoeia has a limited shelf-life of 2 days [10, 15], it was decided to study test kit stability. The parameters of physical stability (colour, fragility) and functional stability (possibility of application (identification of sulfanilamide preparations in the range of application 35–65 mg/ml)) were investigated. The researche was carried out immediately after manufacturing and within 5 months at intervals of 30 days. During study test kits were compared to the newly manufactured ones. The results of the stability study are given in Table. 4

The results obtained within 5 months of test kits stability studying indicate that these analytical tools are sufficiently stable. The usage this test kits can optimize the time spent pharmacist-analyst associated with the preparation of vanillin reagent.

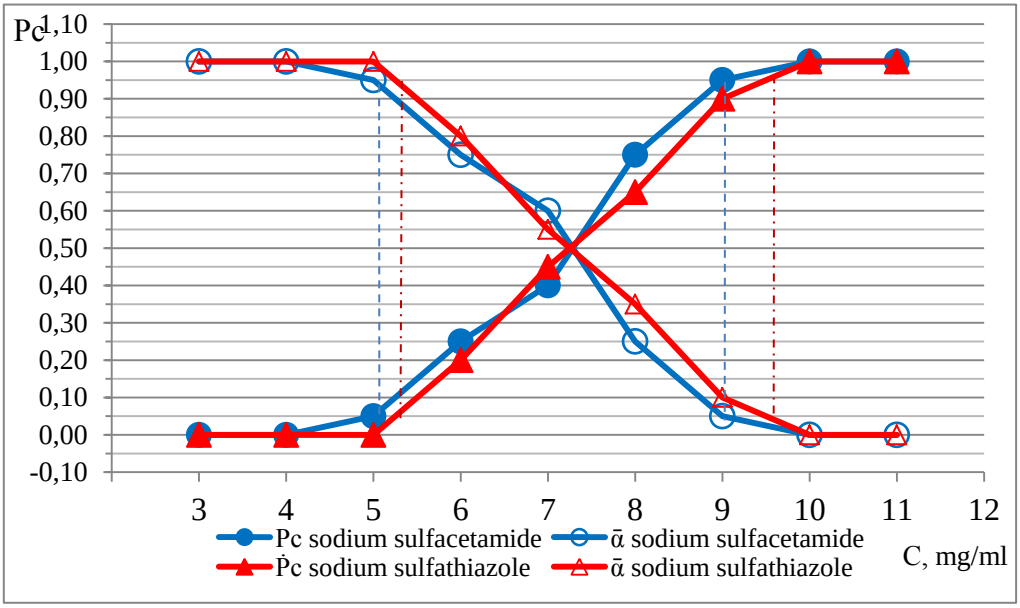

Fig. 1. The "effectiveness curve" for the identification of the derivatives of sulfanilic acid amides using the test kit with vanillin

Table 4

Stability study of the test kits based on FP modified with vanillin

parameter/day	1	30	60	90	120	150
colour	Yes	Yes	Yes	Yes	Yes	Yes
fragility	No	No	No	No	No	No
the possibility of application	+	+	+	+	+	+

7. Conclusions

1. The test kit based on FP, modified with a pharmacopoeia vanillin reagent was developed and tried out in practice for identification of APIs that contain in its structure PAAG;

2. Feasibility of using test kit for the identification of sulfanilamide preparations was confirmed in the range of 35–65 mg/ml;

3. Unreliability intervals of detection of the sodium sulfacetamide and sodium sulfathiazole using the proposed

test kit were investigated; they are 5.0-9.0 mg/ml and 5.3–9.6 mg/ml, respectively;

4. Stability study of the test kit was carried out within 5 months; the results indicate the stability of the test kit and the possibility of its usage during the specified period.

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