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CHROMATOGRAPHIC STUDIES OF DRUG SUBSTANCES: A CASE STUDY IN UNIVERSITY SCIENTIFIC ACTIVITIES

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ABSTRACT

Studying natural sciences at the O.O. Bogomolets National Medical University opens up for students the opportunity to become familiar with advanced scientific discoveries, the prospect of direct participation in conducting experimental research in drug quality control laboratories. The Case study method is used to investigate a problem in a specific context and discuss how the results relate to theory. Case study is actively used in chromatographic studies of medicinal substances, during which ways of solving pharmaceutical analysis problems. The main stages of scientific experimental work according to the requirements of the Case study are determining the purpose of the study with the motivation for the need to carry out this study, motivated selection of samples for the study, drawing up a research plan with a deadline, systematization of the obtained data. In chromatographic studies, an important role is played by the selection of a large number of objects and studies, which provides a high expected increase in information and results. In chemical experiments, it is advisable to use the design of single and multiple studies to compare the results obtained with standards and the design of establishing "anomalies" to register the formation of substances. During chromatographic studies of each specific pharmaceutical object, complex approaches are developed in the analysis of substances, since similar trends are often observed in chromatographic separation, the formation of chemical degradation products. The current task remains the implementation of the Case Study learning model in the performance of scientific work by students by HPLC method.

Key words

Case study, academic research, higher education, university, case study, HPLC, medicinal substances.

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INTRODUCTION

Among the natural sciences studied at the Faculty of Pharmacy of the O.O. Bogomolets National Medical University, the discipline "Pharmaceutical Chemistry" occupies an important place. This discipline provides students with the opportunity to become familiar with advanced scientific discoveries in the field of developing new medicines and the features of pharmaceutical analysis of medicines and their substances of various chemical origins. Students gain the prospect of direct participation in conducting experimental research in drug quality control laboratories and performing scientific experimental work. The case study method is used to investigate a problem in a specific context and discuss how the results relate to theory. Case study is actively used in performing chromatographic studies of medicinal substances, during which solutions to pharmaceutical analysis problems and recommendations for further research are developed - descriptive and problem cases. During chromatographic studies of each specific pharmaceutical object, complex approaches are developed in the analysis of biologically active substances of certain chemical classes, since similar trends are observed in the features of achieving the necessary chromatographic separation, the formation of chemical degradation products, and the detection of specified and unspecified impurities and accompanying substances. Therefore, the current task remains the implementation of the Case Study learning model in the performance of scientific experimental work by students using high-performance liquid chromatography, which gives us the opportunity to develop comprehensive approaches in the analysis of certain classes of drugs, taking into account the peculiarities of this analysis.

LITERATURE REVIEW

The Case study method helps develop critical thinking and is a type of problem-oriented learning. It allows you to implement the task of modeling an experiment, offering non-standard approaches to solving a problem, and finding ways to solve the task. Case studies are used in academia because they are not just a detailed analysis of a particular phenomenon or process, but a broader approach to the study of an object (Ridder, 2017; Rolls, 2013; Seawright & Gerring, 2008; Sikora, 1999). The study has a more standardized structure and uses more rigorous and systematic research methodologies - experiments and analysis. A case study can be used for either a single observation (N=1) or multiple observations (N>1). Due to the specificity of a particular study, the findings cannot be generalized and applied to a broader context. Exploratory research is intended to generalize, and the conclusions can be related to a larger set or scope. Among the types of analysis methods are: illustrative applied research, exploratory applied research, aggregate applied research, descriptive applied research, instrumental applied research (Fig. 1) (Joia, 2002; Koycheva & Yanovskaya, 2023; Pelo et al., 2020; Sheremeta & Kanishchenko, 1999).

Source: Authors.

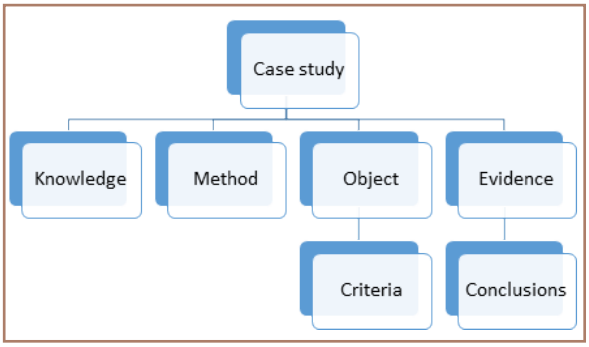

Figure 1. Main components of the Case study

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When using a case study, different research designs can be formed: a design based on methodological work; a research design that emphasizes the difference between single and multiple studies; a "realistic picture" design; a design to detect "anomalies". These designs have significant methodological differences, but are often used in combination. The choice of a research object is often related to a representative sample and different scales of theoretical interests. A representative sample is an average or typical case that does not carry much information. An effective method is to select such a number of studies and objects that will provide a high expected gain in information. The typology of Case study includes the definition of goals (assessment, research), approaches (theoretical justification), choice of processes (single or multiple studies), choice of study (retrospective, diachronic, parallel, sequential) (Hellström et al., 2005; Tsekhmister & Welchinska, 2016; Welchinska & Vilchynska, 2016).

Case studies, as a form of qualitative research, help inform professionals or make evidence-based decisions in a variety of fields. This approach is valuable for health research for developing theory, programs, and planning interventions because of its flexibility and precision.

Qualitative applied research of this method is an approach to research that facilitates the study of a particular phenomenon in its context using a variety of data sources. The question is not examined through a single lens, but rather through a variety of lenses. This approach allows for the discovery and understanding of multiple aspects of the phenomenon.

When planning a study, it is necessary to formulate a research question and understand the case design. It is necessary to understand what the unit of analysis (case) is. Answers to these questions can be effective strategies for further delineating the case.

After deciding whether a qualitative case study is best for the work being done, it is necessary to determine the type of case study. The choice of a specific type of case study design depends on the overall purpose of the study. Case studies are classified as explanatory, exploratory, or descriptive. There is also a distinction between individual, holistic case studies and multiple case studies. Case studies can be internal, instrumental or collective.

Explanatory Case study. Used in situations where it is necessary to explain the alleged cause-and-effect relationships in real-life processes. Such problems are too complex for survey, experimental strategies.

Case study exploratory. Used to investigate situations where the study being evaluated does not have a single set of results.

Case study descriptive. Used to describe a study in real time.

Multi-Case study. Multiple experiments allow the researcher to identify differences within and between experiments. The goal is to replicate findings across experiments. Experiments are compared, cases are carefully selected to predict similar results across experiments, and to predict opposite results based on theory.

Internal Case study. Using the term "internal" implies that the researcher has a strong interest in understanding the problem and aims to better understand the case. It is about a particular feature or problem that is of particular interest to the researcher.

Instrumental Case study. Used to achieve understanding not of a specific situation at the moment, but of those situations that may occur. This helps to understand the problem and improve the theory. The experiment is of

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secondary interest, playing a supporting role, but facilitating the understanding of another experiment. Such an experiment is considered in depth, described in detail. This helps the researcher pursue external interests.

Collective Case study. Collective case studies are similar to multiple case studies.

Single Case study. A specific type of case study and experiment are identified, and the feasibility of conducting a separate case study is considered. The question is whether understanding of the process can be gained by conducting multiple case studies.

Single Case study with Built-in Units. Solving a single experimental problem while conducting a holistic thematic study with built-in units makes it possible to study the experiment, taking into account the experience and capabilities of other laboratories and departments.

Data can be analyzed within the units separately - this is an analysis within the analysis, between different units - this is an analysis between different experiments, across all subunits - this is a cross-experiment analysis. The so-called "saturated analysis" is performed, which contributes to better coverage of the problem. The mistake is that sometimes researchers analyze at the level of an individual subunit, without returning to the global problem that needed to be solved.

Multi-Case study. If the study contains more than one experiment, then it is necessary to conduct a study of several experiments. A multi- or collective case study will allow for the analysis of each experiment and analysis between experiments. A holistic case study with embedded units allows for the understanding of only one unique/critical process. A multiple experiment study examines several procedures or reactions to understand their similarities or differences. Multiple case studies are performed to predict similar outcomes (replication), contrasting outcomes, or presumed causes. The evidence obtained from this study is considered reliable, but can be time-consuming to conduct.

When formulating specific objectives when planning a thematic study, the likelihood of the researcher setting limits on the scope of the study and the possibility of completing the study increase automatically. Conducting a study with a large number of proposals for its planning and stages of implementation always remains within acceptable limits. Proposals can come from laboratory management, research client, scientific supervisor, arise from new ideas and from personal/professional experience, based on theories and the need for their confirmation (Joia, 2002; Pelo et al., 2020).

OBJECTIVES

To introduce basic approaches of the Case study method into complex chromatographic studies using high-performance liquid chromatography (HPLC) of medicinal substances during experimental studies by students *in order* to investigate alternative conditions for HPLC chromatography of the studied samples, which could demonstrate higher identification ability when determining their purity and modifying research methods with optimal conditions for protecting molecules from chemical degradation.

METHODOLOGY

The main stages of scientific experimental work according to the requirements of the Case study are determining the purpose of the study with the motivation for the need to perform a specific study, motivated selection of samples for the study (purposeful, random, valuable for the study), consultation with the

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scientific supervisor and experts, drawing up a research plan with a deadline, systematizing the data obtained (primary, secondary, statistical), identifying 5-6 key strengths and weaknesses of the study using SWOT analysis to predict potential threats to the experiment and unplanned changes in the work strategy, taking into account the limitations of the method for a specific experiment, setting the limits of the planned study, outlining possible alternative solutions, replacing irrational approaches to performing the planned study. A serious drawback of pharmaceutical analysis of drugs and their substances is the lack of many parameters of their standardization, which must meet the requirements of GLP and GMP.

The State Pharmacopoeia of Ukraine (SPU) does not regulate the analysis of a certain number of substances and medicinal products. In addition, accompanying substances, specified and unspecified impurities, as well as related substances in the test samples, according to the recommendations of the SPU and the European Pharmacopoeia (Eur.Ph.), are analyzed only using the liquid chromatography (LC) method. The choice of the HPLC method for studying medicinal substances is justified by its modernity and high selectivity in studying the purity and integrity of medicinal substances, as it allows to increase the efficiency and effectiveness of their analysis. Experimental studies were performed on an Agilent 1260 Infinity II chromatograph with a UV detector, on INERTSIL ODS-3V, ZORBAX Eclipse Plus C18 columns. Pharmacopoeial standard samples (PSS) of SPU chlorhexidine, sodium formaldehyde sulfoxylate dihydrate (rongalite) and samples of the studied substances chlorhexidine, sodium metamizole monohydrate were used. Computer analysis was performed using the OpenLab CDS program.

RESULTS AND DISCUSSION

According to the typology of Case study, we used the design of single and multiple studies – chromatographic study by HPLC of the substances chlorhexidine and metamizole sodium, each of 5 samples from different batches and at 5 punctures and the design of detecting "anomalies" – identification and quantitative determination of accompanying impurities, unacceptable impurities, which can be represented by intermediates of synthesis of substances, products of chemical degradation of starting molecules and related substances. The selection of a large number of objects and their studies provided a high expected increase in information and results. The goal was set to adapt chromatography conditions and HPLC research methods for assessing the purity and identification and quantification of active pharmaceutical ingredients (API) of chlorhexidine and metamizole sodium as an alternative analysis method. Theoretical justification – introduction into pharmaceutical analysis of a modern highly selective HPLC method with higher identification ability as an alternative to the pharmacopoeial method of HPLC research, modification of chromatography conditions and research methods to create optimal conditions for protection against chemical degradation of the structure of substance molecules.

Process selection – multiple studies that allow accumulating results and analyzing them. Research selection – parallel studies with comparison of results and sequential studies were conducted.

The substances chlorhexidine and metamizole sodium were chosen as the objects of the study, which were to become the basis for the creation of dosage forms of medicinal products (Chlorhexidine (Oral Route) Precautions -

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Mayo Clinic, 2023; Muniz et al., 2023; Wade et al., 2021; Wade et al., 2021). Chlorhexidine is used in pharmacy in the form of salts - gluconate, digluconate, acetate or diacetate, as liquids or powders. Chlorhexidine molecules are based on a bis-condensed system consisting of biguanide, hexamethylene, 4-chlorophenyl fragments.

When ingested, chlorhexidine is poorly absorbed from the gastrointestinal tract, causing stomach irritation and nausea. Aspiration of chlorhexidine into the lungs can be fatal due to the high risk of acute respiratory distress syndrome. Chlorhexidine acetate is used as a general antiseptic in 0.05% and 0.1% solutions. It is used for cleaning and disinfecting wounds, for antiseptic treatment of burns (Picoli et al., 2022; Ribas et al., 2020; Soares et al., 2019). Chlorhexidine digluconate is a broad-spectrum antiseptic. It can be safely used in low concentrations in mouthwashes and contact lens solutions. Chlorhexidine gluconate is used as a mouthwash to treat gingivitis (swelling, redness, and bleeding of the gums). This drug is prescribed by a dentist. The bactericidal efficacy of guanide, biguanide, and bisbiguanide agents has been studied on intact microorganisms: *Streptococcus mutans, S. sanguis, Actinomyces viscosus*, and *A. naeslundii.* The pharmacological activity of the compounds was studied based on the features of their molecular configuration. Studies have shown that bis- and biguanide configurations are more effective. They have alkyl side chains of sufficient length in the molecule. At the same time, no single structural fragment has the effectiveness (Di Paolo et al., 2021; Lee et al., 2019; Villa et al., 2018).

Chlorhexidine is determined by a colorimetric method. The method is based on the formation of a yellow complex between chlorhexidine and bromocresol green, which is isolated by chloroform extraction. The absorption peak of this complex is at 410 nm. A linear response is achieved from 2.5 to 30 µg chlorhexidine/ml. The accuracy of this method makes it useful for the determination of chlorhexidine in pharmaceutical analysis of pharmaceutical mixtures.

The spectrophotometric determination of chlorhexidine is performed using a liquid-liquid extraction method using bromophenol blue. The chemical structure of chlorhexidine is determined using a combination of single crystal X-ray diffraction (SC-XRD), electrospray ionization mass spectrometry (ESI-MS), 1H nuclear magnetic resonance (NMR) spectroscopy, correlation spectroscopy (COSY) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Also, an analytical technique based on the Box-Behnken design and using the HPLC method is used, which helps to quantitatively determine chlorhexidine and thymol simultaneously in a matrix with pharmaceutical excipients (Chandran et al., 2022; Sokolik et al., 2018; Vrachas et al., 2022).

SPU does not regulate the analysis of chlorhexidine substance. Pharmaceutical analysis is performed according to the European Pharmacopoeia (European Pharmacopoeia (11-th ed.), 2022) of medicinal products: Chlorhexidine diacetate, Chlorhexidine digluconate solution, Chlorhexidine dihydrochloride. Accompanying substances are examined by the LC method (2.2.29).

The substance is dissolved in a mixture of solvents: trifluoroacetic acid *P* - acetonitrile *P* - trifluoroacetic acid *P* in water for chromatography or alternatively: trifluoroacetic acid *P* - trifluoroacetic acid *P* in water for chromatography *P* - trifluoroacetic acid *P* in acetonitrile *P*.

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Among the specified and unspecified impurities of the substance regulated by the Pharmacopoeia, there are 16 substances: A, B, C, D, E, F, G, H, I, J, K, L, N, O, P, Q. Impurity P (chloroaniline) is a specified impurity. Impurity Q refers to unidentified impurities - substances with an unknown structure. Among the specified and unspecified impurities of the substance regulated by the Pharmacopoeia, there are 16 substances: A, B, C, D, E, F, G, H, I, J, K, L, N, O, P, Q. Impurity P (chloroaniline) is a specified impurity. Impurity Q refers to unidentified impurities - substances with an unknown structure.

We performed a chromatographic study of the chlorhexidine substance under the following chromatography conditions: column – INERTSIL ODS-3V with a temperature of 25˚C, UV detection at 254 nm, flow rate of 1.0 ml/min, injection volume of 10 µl, chromatography time of 60 min.

The following mobile phases were used: mobile phase A – a mixture of solvents for HPLC: trifluoroacetic acid *P* – acetonitrile *P* – trifluoroacetic acid *P* in water for chromatography, 0.1% *P* (20:20:80, V/V/V) and mobile phase B – a mixture of solvents for HPLC: trifluoroacetic acid *P* – trifluoroacetic acid *P* in water for chromatography *P* – trifluoroacetic acid *P* in acetonitrile *P* (10:10:90, V/V/V).

A gradient method was used to obtain separation and identification of a larger number of impurities (Table 1).

When studying the PSS and test samples after 5 stabs, the following results were obtained (Tables 2, 3, Fig. 2).

Table 1. Gradients

Source: Authors

Time (min)	Mobile phase A (%, V/V)	Mobile phase B (%, V/V)
0-2	100	0
2-32	80	20
32-47	80	20
37-47	70	30
47-54	70	30

Table 2. Results of chromatography of chlorhexidine PSS

Source: Authors

	PSS 1		PSS 2	
	Chlorhexidine			
1	2	3	4	5
	RT	Area	RT	Area
	2,901	59,837	2,897	62,047
	2,917	61,500	2,914	63,333
	2,917	61,440	2,911	62,680
	2,916	59,467	2,899	61,999
	2,902	60,900	2,897	61,985
Average	2,914	60,268	2.909	62,690

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Continuation of table 2

1	2	3	4	5
SD	0,093	1,083	0,070	0,909
RSD (≤2.0%)	0,38	1,80	0,30	1,45

Note: RT – retention time (min), Area – peak area, SD – standard deviation, used to determine RSD), RSD – relative standard deviation, according to the Pharmacopoeia, should be less than 2)

Table 3. Chromatography results of the tested chlorhexidine samples

Source: Authors

	Sample 1			Sample 2	
	Chlorhexidine RT	*Impurity P RT*	*Impurity 1 RT*	*Chlorhexidine RT*	*Impurity P RT*
	2,818	4,401	5,090	2,890	4,347
	2,919	4,300	5,260	2,914	4,333
	2,380	4,267	5,198	2,911	4,280
	2,916	4,465	5,199	2,899	4,099
	2,900	4,302	5,200	2,897	4,185
Average	2,786	4,347	5,189	2.902	4,248
SD	0,088	1,053	1,066	0,070	1,052
RSD (≤2.0%)	0,36	1,70	1,57	0,31	1,69

Note: RT – retention time (min), SD – standard deviation, used to determine RSD), RSD – relative standard deviation, according to the Pharmacopoeia, should be less than 2).

Source: Authors

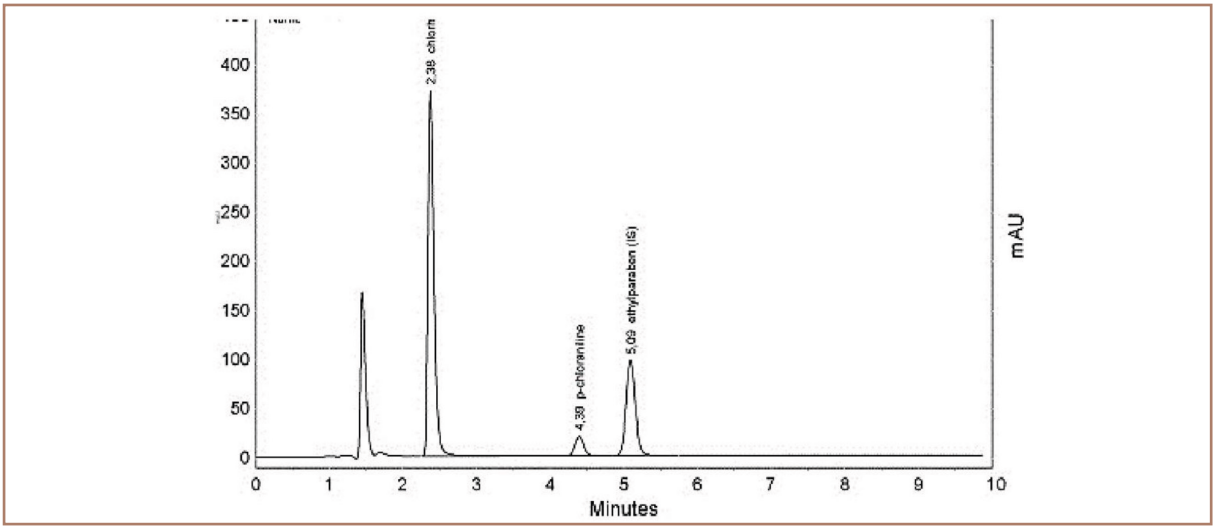

Figure 2. Chromatogram of test sample 1: chlorhexidine (Rt=2.380 min), specified impurity P (para-chloroaniline) (Rt=4.300 min), unacceptable impurity 1 (ethylparaben) (Rt=5.090 min)

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Metamizole sodium monohydrate is a well-known drug with analgesic, antipyretic and anti-inflammatory effects. Under certain conditions, this drug can cause undesirable effects, such as Quincke's edema, anaphylactic shock, Stevens-Johnson syndrome. It leads to the development of agranulocytosis, aplastic anemia, neutropenia and pancytopenia (European Medicines Agency (EMA), 2019; Rudin et al., 2020).

According to the SPU (Derzhavna Farmakopeya Ukrayiny, 2014) the identification of the substance metamizole sodium is performed by absorption spectrophotometry in the IR region, chemical methods, and tests for the transparency of the substance solution are performed (2.2.1).

Accompanying impurities are detected by the LC method (2.2.29) with UV detection at 254 nm: specified impurities C and E, unspecified impurities A, B, D. The test solution, reference solutions a, b, d, e are prepared in methanol, solution c – in methanol and menthol. The UV spectrum of metamizole sodium (in an acidic medium) contains an absorption maximum λmax = 258 nm.

According to the SPU, the purity of metamizole sodium is determined by the LC method. Accompanying substances are identified with UV detection at 254 nm: specified impurities include impurities C and E, unspecified impurities include A, B, D. We performed a chromatographic study of the substance metamizole sodium under the following chromatography conditions: column – ZORBAX Eclipse Plus C18 with a temperature of 25˚C, UV detection at 215 nm, flow rate 1.0 ml/min, injection volume 5 µl, chromatography time 13 min. The mobile phases used were: mobile phase A (3.2 g of triethylamine in 1000 ml of water, pH of the solution 3.0±0.05 using phosphoric acid), mobile phase B – methanol.

A gradient method was used to obtain separation and identification of impurities (Table 4).

Table 4. Gradients

Source: Authors

Time (min)	Mobile phase *A*, (%, V/V)	Mobile phase *B*, (%, V/V)
0,0	100	0
3,5	100	0
5,5	50	50
7,5	50	50
10,0	100	0
13,0	100	0

Sodium formaldehyde sulfoxylate dihydrate (rongalite) was used as the PSS of the SPU. Formaldehyde sulfoxylate dihydrate or sodium formaldehyde sulfoxylate (rongalite) is a chemical substance that is dangerous to the human body (Chen et al., 2020; Golla et al., 2020; He et al., 2021). It can be formed during the synthesis of the substance, so it is important to check its purity for the presence of rongalite.

When studying the PSS and test samples after 5 punctures, the following results were obtained (Tables 5, 6; Fig. 3).

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Table 5. Results of chromatography of the PSS of rongalite

Source: Authors

	PSS 1			PSS 2		
		Rongalite				
	RT	*Area*	*T (≤2.0)*	*RT*	*Area*	*T (≤2.0)*
	1,990	259,483	1,1	1,992	258,158	1,1
	1,993	258,894	1,1	1,993	260,702	1,1
	1,990	258,950	1,1	1,911	258,160	1,1
	1,994	258,473	1,1	1,899	259,333	1,1
	1,990	258,359	1,1	1,992	258,230	1,1
Average	1,991	258,832	1,1	1.993	259,430	1,1
SD	0,002	0,446		0,001	1,799	
RSD (≤2.0%)	0,10	0,17		0,04	0,69	

Note: RT – retention time (min), Area – peak area, SD – standard deviation, used to determine RSD), RSD – relative standard deviation, according to the Pharmacopoeia, should be less than 2), T-tailing (peak shape, often less than 2).

Table 6. Results of chromatography of tested samples of metamizole sodium for the presence of rongalite

Source: Authors

	Sample 1		Sample 2	
	Rongalite		*Rongalite*	
	RT	*Area*	*RT*	*Area*
	1,983	40,032	1,999	42,435
	2,009	40,692	1,997	44,148
	2,003	38,371	2,003	41,287
	1,918	39,008	2,001	41,300
	1,997	39,015	2,000	42,433
Average	1,998	39,698	2.000	42,623
SD	0,002	0,442	0,002	1,450
RSD (≤2.0%)	0,11	0,23	0,02	0,38

Note: RT – retention time (min), Area – peak area, SD – standard deviation, used to determine RSD), RSD – relative standard deviation, according to the Pharmacopoeia, should be less than 2).

Source: Authors

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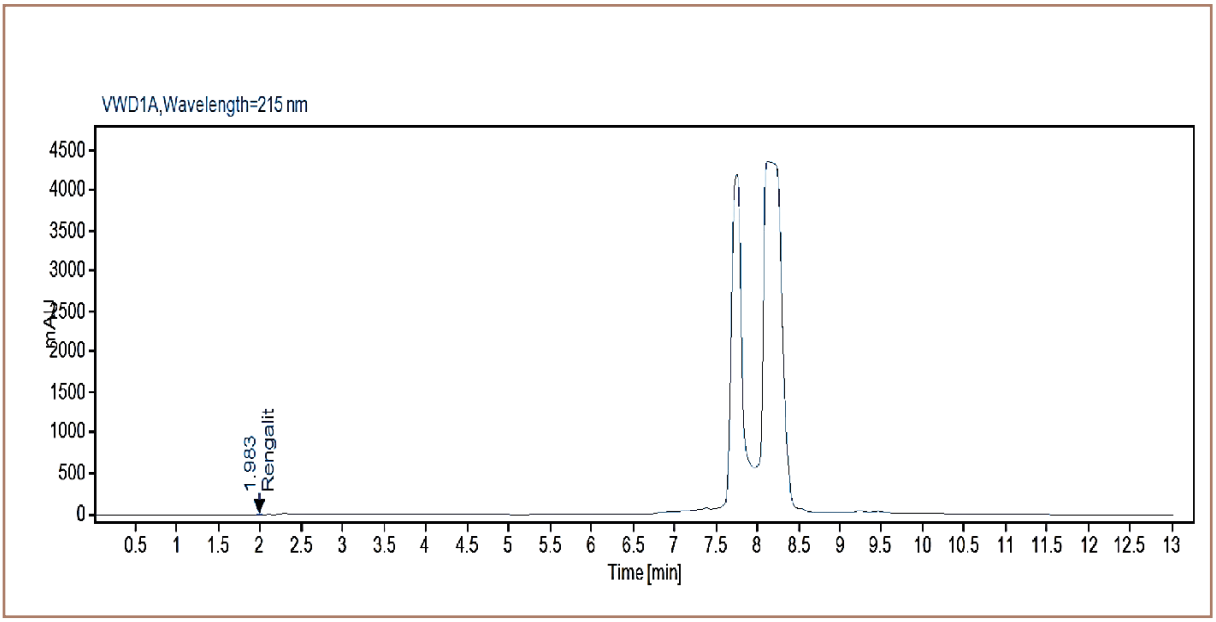

Figure 3. Chromatogram of the studied sample of metamizole sodium substance with an admixture of rongalite (HPLC method): Rt (rongalite) = 1.983 min

Thus, the presence of sodium formaldehyde sulfoxylate dihydrate (rongalite) – an unacceptable impurity – was chromatographically confirmed: Rt in the range of 1.918-2.009 min (at 215 nm).

A shift of the rongalite peak in the test substance is observed compared to standard rongalite samples (Rt 1.899-1.993 min, at 215 nm), which can be explained by the presence of metamizole sodium in the test substance.

A laboratory report is compiled based on the results of the research. It includes the following sections:

- introduction, formulation of the goal, hypothesis or task of the research;
- justification of the method (methods) used to perform the tasks set, methods of processing the obtained data;
- results, description of the data obtained, characteristics of products, characteristics of certain physicochemical parameters or properties of substances;
- discussion of the results obtained, which correspond or do not correspond to the purpose and hypothesis that were the reason for the start of the research;
- conclusion about the overall result of the practical work, how the conclusions correlate with the larger body of scientific knowledge.

Laboratory report of the performed chromatographic study according to the Case study typology.

Introduction: using the design of single and multiple studies and the design of detecting "anomalies", it is planned to conduct chromatographic studies by HPLC of the substances chlorhexidine and metamizole sodium (each of 5 samples from different batches and at 5 punctures) in order to adapt the chromatography conditions and the HPLC research methodology for the assessment of purity, identification and quantification of active pharmaceutical ingredients (API) of

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chlorhexidine and metamizole sodium as an alternative to the pharmacopoeial method of LC analysis.

Justification of the method. Introduction of a modern highly selective HPLC method with higher identification ability into pharmaceutical analysis as an alternative to the pharmacopoeial method of LC research, modification of chromatography conditions and research methods to create optimal conditions for protection against chemical degradation of the structure of substance molecules and a high degree of chromatographic separation of the components of the tested substances. The studies were conducted multiple times with the accumulation of results and analysis of the data obtained by comparison with the results obtained during the study of the PSS. and analyze them. Parallel and sequential studies were performed with the comparison of the results.

Results. HPLC chromatographic identification of API, quantitative determination of API and accompanying substances (specified and unspecified impurities, unacceptable impurities) was performed. The purity of the tested substances was determined. The selection of a large number of objects and their studies provided a high expected increase in information and results.

Discussion of the obtained results. During the chromatographic study of chlorhexidine substances by HPLC, in addition to the specified impurity P (para-chloroaniline) (Rt=4.300 min), an unacceptable impurity 1 (ethylparaben) (Rt=5.090 min) was identified.

Ethylparaben or methyl ester of para-hydroxybenzoic acid is used as a food additive E218 as a preservative and as an antiseptic.

When analyzing the chromatographic data and comparing the location of the peaks, their shifts were detected on the chromatograms of the tested chlorhexidine samples Rt from 2.380 min to 2.914 min (average Rt value from 2.786 min to 2.902 min) in comparison with the location of the chlorhexidine peaks on the chromatograms of the PSS Rt from 2.897 min to 2.917 min (average Rt value from 2.909 min to 2.914 min).

This can be explained by the presence of the impurity P (para-chloraniline) and the unacceptable impurity ethylparaben in the tested substances of chlorhexidine. The presence of sodium formaldehyde sulfoxylate dihydrate (rongalite) - an unacceptable impurity - was chromatographically confirmed in the composition of the tested substances of metamizole sodium: Rt from 1.918 min to 2.009 min. (at 215 nm).

There is a shift in the peak of rongalite in the composition of the test substance compared to the standard samples of rongalite (Rt from 1.899 min to 1.993 min, at 215 nm), which can be explained by the presence of metamizole sodium in the composition of the test substance. Rongalite is often formed as an intermediate product during the synthesis of the substance, is a toxic substance, therefore it is an unacceptable impurity.

Conclusion on the overall result of the research work. The design of single and multiple studies and the design of detecting “anomalies” by conducting parallel and sequential studies with a comparison of the results of an alternative pharmacopoeial method - a high-tech HPLC method for studying the test substances chlorhexidine and metamizole sodium - are proposed.

The HPLC method was tested in practice and found to provide higher identification capability compared to the liquid chromatography method, and also allows for in-depth pharmaceutical analysis of the studied objects in order to establish their quality.

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CONCLUSIONS

1. Using the Case study method, we have built an algorithm for performing chromatographic studies by HPLC of the tested medicinal substances chlorhexidine and metamizole sodium using the design of single and multiple studies and identifying "anomalies" and conducting parallel/sequential studies with a comparison of the results obtained.
2. The selection of a large number of objects – substances chlorhexidine and metamizole sodium (each from 5 samples from different batches and with 5 punctures, a total of 50 results) provided a high expected increase in information and results.
3. Chromatography conditions and research methods were adapted using HPLC, a method with high identification capacity for assessing the purity, identification and quantification of active pharmaceutical ingredients (API) of chlorhexidine and metamizole sodium substances and detecting accompanying substances in the composition of the substances.
4. During the chromatographic study of chlorhexidine substances by HPLC, an unacceptable impurity 1 (ethylparaben) (Rt=5.090 min) was identified, the presence of which likely affects the shifts of chlorhexidine peaks on the chromatograms of its tested samples in comparison with the location of chlorhexidine peaks in the composition of the PSS. In the composition of the tested substances of metamizole sodium, the presence of sodium formaldehyde sulfoxylate dihydrate (rongalite) - a toxic unacceptable impurity - was chromatographically confirmed, and a shift of the rongalite peak in the composition of the tested substance was also observed compared to the location of its peak in the composition of the PSS.

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BIBLIOGRAPHIC REFERENCES

1. Chandran, P., Pillai, S. C., Govindasamy, S., Arumugasamy, S. (2022). Detection of the Release of Chlorhexidine from Cured Denture Resins Discs: Subsequently Deducing the Ability of Denture Resin as a Drug Carrier. *JDMT* , 11, 127–137. DOI: 10.22038/JDMT.2022.59581.1465.
2. Chen, X.-L., Tang, B.-C., He, C., Ma, J.-T., Zhuang, S.-Y., Wu, Y.-D. and Wu. A.-X. (2020). Rongalite as a sulfone source: a novel copper-catalyzed sulfur dioxide anion incorporation process. *Chemical Communications*, 56, 13653-13656.
3. Chlorhexidine (Oral Route) Precautions - Mayo Clinic. (2023) www.mayoclinic.org
4. Derzhavna Farmakopeya Ukrayiny (State Pharmacopoeia of Ukraine) (2-nd ed.). (2014). Derzhavne pidpryyemstvo "Ukrayins'kyy naukovyy farmakopeynyy tsentr yakosti likars'kykh zasobiv" (State enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products"). Kharkiv: Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products, 2, 442-444 (in Ukrainian).

5. Di Paolo, G. B., Pereira, C. S., Souza Júnior, A. R., Machado, F. C., Carvalho, T. d A. (2021). Impacts of Intensive Care Patients' Oral Hygiene on Nosocomial and Ventilator-Associated Pneumonia: Integrative Literature Review. *Research, Society and Development,* 10, e376101321586. DOI: 10.33448/rsd-v10i13.21586.
6. European Medicines Agency (EMA). (2019). Metamizole containing medicinal products. DOI: https://www.ema.europa.eu/en/medicines/human/referrals/metamizole-containing-medicinal-products.
7. European Pharmacopoeia (11-th ed.). (2022). Council of Europe, Strasbourg: EDQM, 2, 2295-2301.
8. Golla, S., Anugu, N., Jalagam, S. and Kokatla, H.P. (2020). Rongalite-induced transition-metal and hydride-free reductive aldol reaction: a rapid access to 3,3'-disubstituted oxindoles and its mechanistic studies. *Organic & Biomolecular Chemistry*, 20**,** 808-816.
9. He, F.-S., Zhang, M., Zhang, M., Luo, X. and Wu, J. (2021). Iminyl radical initiated sulfonylation of alkenes with rongalite under photoredox conditions. *Organic Chemistry Frontiers,* 8**,** 3746-3751.
10. Hellström I., Nolan M., Lundh U. (2005). 'We do things together': A case study of 'couplehood' in dementia. *Dementia*, 4(1), 7-22. DOI:10.1177/1471301205049188.
11. Joia (2002). Analysing a web-based e-commerce learning community: A case study in Brazil. *Internet Research*, 12, 305-317. DOI:10.1108/10662240210438434.
12. Koycheva, O.S. & Yanovskaya, L.G. (2023). Vykorystannya metodu Keys vyvchennya yak analizu problemnykh sytuatsiy v istorychniy osviti (Using the Case-study method as an analysis of problem situations in historical education). *Innovatsiyna pedahohika – Innovative pedagogy. 58(1),* 77-80 (in Ukrainian).
13. Lee, S., Laghapour Lighvan, N., McCredie, V., Pechlivanoglou, P., Krahn, M., Quiñonez, C., Azarpazhooh, A. (2019). Chlorhexidine-Related Mortality Rate in Critically Ill Subjects in Intensive Care Units: A Systematic Review and Meta-Analysis. *Respiratory Care*, 64, 337–349. DOI: 10.4187/respcare.06434.
14. Muniz, V.M., Júnior, J.V. C., Aragão, C.F.S., de Souza, F., Sampaio, F.C. (2023). A HPLC method for simultaneous quantification of chlorhexidine and thymol using Box-Behnken design for robustness of the method assessment. *Journal of Liquid Chromatography & Related Technologies,* 46, 6-10, 168-179.
15. Pelo, I.M., Reva, T.D., Nizenkovska, I.V., Kozak, N.D., Konovalova, L.V. (2020). Trends in the professional training of pharmacy specialists in Ukraine. *Medicni Perspektivi*. 25(3), 4-8. DOI: 10.26641/2307-0404.2020.3.214543.
16. Picoli, M. E. F. da S., Botelho, M. C. P., Ananias, F., Secco, A. S. (2022). Evaluation of Chlorhexidine Efficiency in Reduction of Microbial Load in Water Lines of Dental Equipment. *Brazilian Journal of Development,* 8, 23074–23095. DOI: 10.34117/bjdv8n4-022.
17. Ribas, M. A., de, L., Santos, B. M., Botelho, M. P. J. (2020). Evaluation of the Bactericide Property of 0.12% and 0.2% Chlorexidine Digluconate in Solution. *Brazilian Journal of Development,* 6, 4621–4634. DOI: 10.34117/bjdv6n1-331.
18. Ridder, H.G. (2017). The theory contribution of case study research designs. *Business Research.* 10, 281–305.DOI: https://doi.org/10.1007/s40685-017-0045-z.
19. Rolls, G. Classic Case Studies in Psychology. (2-nd ed.) (2013). Abingdon, England: Hodder Education, 24-110. DOI: https://doi.org/10.4324/9780203783900.
20. Rudin, D., Schmutz, M., Roos, N. J., Bouitbir, J., Krähenbühl, S. (2020). Reactive Metamizole Metabolites Enhance the Toxicity of Hemin on the ATP Pool in HL60 Cells by Inhibition of Glycolysis. *Biomedicines*, 8(7), 212. DOI: https://doi.org/10.3390/biomedicines8070212.
21. Seawright, J. & Gerring, J. (2008). Case Selection Techniques in Case Study Research: A Menu of Qualitative and Quantitative Options. *Political Research Quarterly*, 61(2), 294-308. DOI: https://doi.org/10.1177/1065912907313077.
22. Sheremeta, P. M. & Kanishchenko, L. G. (1999). *Keys-metod: z dosvidu vykladannya v ukrayins'kiy biznes-shkoli (Case method: from the experience of teaching at a Ukrainian business school).* 2-nd ed. Kyyiv: Tsentr innovatsiy ta rozvytku, 80 p. (in Ukrainian).
23. Sikora, Ya. B. (1999). Keys-tekhnolohiyi pry vyvchenni "Metodiv optymizatsiyi" (Case technologies in the study of "Optimization Methods"). Proceedings from: *Naukovo-doslidna robota molodykh uchenykh: stan, problemy, perspektyvy: Materialy II Vseukrayins'koyi naukovo-praktychnoyi internet-konferentsiyi, prysvyachenoyi 95-richchyu Khersons'koho derzhavnoho universytetu – Research work of young scientists: status, problems, prospects: Materials*

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ISSN 3068-3580
https://www.us-journal.us

of the II All-Ukrainian scientific and practical Internet conference dedicated to the 95th anniversary of Kherson State University. (pp.244-248). Kherson (in Ukrainian).

24. Soares, A. K., Bonvini, B., Fukushigue, C. Y. (2019). Evaluation of the Prophylactic Antimicrobial Potential of Mouthwashes Containing in Their Formulation Chlorhexidine and Essential Oils. *Salusvita*, *38*, 87–96.
25. Sokolik, C. G., Ben-Shabat-Binyamini, R., Gedanken, A., Lellouche, J. P. (2018). Proteinaceous Microspheres as a Delivery System for Carvacrol and Thymol in Antibacterial Applications. *Ultrasonics Sonochemystry,* 41, 288–296. DOI: 10.1016/j.ultsonch.2017.09.032.
26. Tsekhmister, Y. & Welchinska, E. (2016). Carbon (II) monoxide as a subject for poisons studying of study on discipline toxicological chemistry (criminal analysis) at the pharmaceutical department of the National Medical University in Ukraine. *CBU International conference proceedings 2016: Innovations in Science and Education*, 4, 744-749. DOI 10.12955/cbup.v4.843.
27. Villa, O., Ramberg, P., Fukui, H., Emilson, C. G., Papanikolaou, G., Heijl, L., Birkhed, D. (2018). Interaction between Chlorhexidine and Fluoride in a Mouthrinse Solution—A 4-Day and 6-Week Randomized Clinical Pilot Study. *Clinical Oral Investigations,* 22, 1439–1448. DOI: 10.1007/s00784-017-2219-7.
28. Vrachas, A., Gkountanas, K., Boutsikaris, H., Dotsikas, Y. (2022). Development and Validation of a Novel RP-HPLC Method for the Determination of Cetrimide and Chlorhexidine Gluconate in Antiseptic Solution. *Analytica*, 3, 79–91.DOI: 10.3390/analytica3010006.
29. Wade, R.G., Bourke, G., Wormald, J.C., Totty, J.P., Stanley, G.H., Lewandowski, A. et al. (2021). Chlorhexidine versus povidone-iodine skin antisepsis before upper limb surgery (CIPHUR): an international multicentre prospective cohort study". *BJS Open*, 5(6): zrab117. DOI:10.1093/bjsopen/zrab117.
30. Wade, R.G., Burr, N.E., McCauley, G., Bourke, G., Efthimiou, O. (2021). The Comparative Efficacy of Chlorhexidine Gluconate and Povidone-iodine Antiseptics for the Prevention of Infection in Clean Surgery: A Systematic Review and Network Meta-analysis". *Annals of Surgery*, 274 (6): e481e488. DOI:10.1097/SLA.0000000000004076.
31. Welchinska, E. & Vilchynska, V. (2016). New compound N1,N1'-(2"-bromo-2"-chloroethenyl)-bis-(5-fluorouracil) as the active antitumor agent for sarcoma 180. *CBU International conference proceedings 2016: Innovations in Science and Education,* 4, 740-743. DOI 10.12955/cbup.v4.842.

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