BABES – BOLYAI UNIVERSITY,
CLUJ – NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

SUMMARY OF THE DOCTORAL THESIS

STRUCTURE, LIPOPHILICITY AND BIOACTIVITY RELATIONSHIPS IN THE CASE OF ANTIPSYCHOTIC, ADRENERGIC DRUGS AND PLANTS WITH ANTIPSYCHOTIC EFFECTS

Ph. D. student: Druțaș Melinda Carmen (căs. Szasz)
Doctoral advisor: Prof. Dr. Habil. Claudia Cimpoiu (Universitatea Babeș – Bolyai, Cluj – Napoca)

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Keywords: antipsychotic drugs, adrenergic drugs, receptor binding profile, lipophilicity, scor de bioactivity score, active principles with antipsychotic effect, antioxidant activity, ABTS and DPPH detection

INTRODUCTION

Schizophrenia is a neuropsychiatric disorder characterized by a variety of positive, negative and cognitive symptoms including delusions, hallucinations, irrational thinking, aggressive behavior, loss of interest, difficulties in communication, concentration and others. Schizophrenia affects approximately 1% of the population and is in the top 10 causes of disability worldwide. The exact causes of this disorder are not fully elucidated. It is assumed to be the results of the interaction of several factors such as: viral diseases during pregnancy, obstetric complications, severe stress, cannabis use in adolescence by individuals with genetically predisposition and environmental factors.

There is no curative treatment for schizophrenia. Antipsychotic drugs are the main treatment for the improvement of the symptoms of schizophrenia and have severe limitations due to mainly improving positive symptoms, negative and cognitive symptoms remain untreated. Another important cause of low adherence to antipsychotic treatment is due to the significant side effects. Antipsychotic drugs show significant variability in the interaction with the neurotransmitters, which is reflected in their therapeutic efficacy and specific side effects profile. This variability contributes to their classification into first-generation (typical) and second-generation (atypical) antipsychotic drugs.

Typical antipsychotic drugs improve positive symptoms and produce irreversible extrapyramidal side effects. Atypical antipsychotic drugs improve positive and negative symptoms, have a low risk of extrapyramidal side effects, but can produce significant side effects such as metabolic side effects, sexual dysfunction or lethal side effects (through agranulocytosis, in the case of clozapine if hematological parameters are not properly monitored).

The study of the receptor binding profiles bioactivity and lipophilicity of antipsychotic drugs is important for obtaining an overview of antipsychotic drug treatment.

Considering the existing deficiencies in the treatment with antipsychotic drugs, it is necessary to research and identify some solutions to improve the symptoms of schizophrenia and reduce the adverse effects. Medicinal plants represent a valuable source of active principles that could be used to optimize the treatment of mental disorders.

Development of rapid chromatographic analytical methods for the simultaneous analysis of drugs and medicinal plants.

THE PURPOSE FOR SELECTING THIS TOPIC AND OBJECTIVES

Schizophrenia is a severe mental illness that affects young people, usually starting in late adolescence or early adulthood. Antipsychotic treatment, although essential for the improvement of symptoms, presents a lot of challenges for specialists in the field, such as doctors, pharmacists, nurses, psychologists due to the limitations that these drugs presents in the improving of all the categories of symptoms and the significant side effects.

The medicinal plants represent valuable resources of active principles that associated with antipsychotic treatment could help to improve antipsychotic treatment.

The aim of the research is to investigate the structure, receptor binding profiles, lipophilicity and bioactivity relationship between drugs and medicinal plants with antipsychotic effects, in an interdisciplinary context, which integrates chemistry and pharmacy concepts.

Development and validation of rapid chromatographic methods for the analysis of drugs and medicinal plants.

The general objective of the thesis included three specific objectives. The first specific objective focuses on analyzing the antipsychotic drugs authorized in Romania, in terms of lipophilicity, receptor binding affinity profile, bioactivity, aiming to establish correlation between these parameters to obtain an overall picture.

The second objective consists in the study of medicinal plants, the identification of active principles with antipsychotic effects, the determination of their bioactivity, the discussion of these findings compared with the results of the study of antipsychotic drugs, drawing conclusions and future study perspectives.

The third part focuses on the development of methods for the analysis of drugs and active principles from plants. The initial development of an HPTLC method for the rapid and simultaneous determination of adrenergic drugs banned in sports followed by the adaptation of this newly developed and validated method for the analysis of drugs and plants with antipsychotic effects.

CHAPTER 1. SCHIZOPHRENIA: DEFINITION, SYMPTOMS AND TREATMENT

1.1 GENERAL DESCRIPTION OF SCHIZOPHRENIA

Schizophrenia is a chronic neuropsychiatric disorder with a significant impact on the pacient's quality of life [1]. It is a complex neuropsychiatric disorder that affects approximately 1% of the population [2] and is in the top 10 causes of disability worldwide [3].

Schizophrenia leaves patients with an inability to effectively manage daily tasks, maintain employment, relationships and even self-care [4].

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1.2 CURRENT THERAPEUTIC APPROACHES

Atypical antipsychotics drugs apart from clozapine are the first-line treatment for the improvement of the symptoms of schizophrenia.

Atypical antipsychotics drugs have metabolic adverse effects such as weight gain, hyperlipidemia, diabetes, and may contribute to an increased risk of cardiovascular mortality in patients with schizophrenia [6,7].

1.3 ANTIPSYCHOTIC DRUGS AUTHORIZED IN ROMANIA

Table 1 presents the antipsychotic drugs authorized in Romania for the treatment of schizophrenia and other types of psychosis. Among these drugs are the below representatives:

Table 1. Classification of antipsychotic drugs authorized in Romania by the National Agency of Medicines and Medical Devices (ANMDM)[9,15].

Pharmacological class	Representatives	International common name (DCI)
Typical antipsychotics/ neuroleptics/ (first generation)	Levomepromazine	LEVOMEPROMAZINUM
	Flupentixol	FLUPENTIXOLUM
	Zuclopentixol	ZUCLOPENTHIXOLUM
	Haloperidol	HALOPERIDOLUM
	Sulpiride	SULPIRIDUM
	Amisulpride	AMISULPRIDUM

Pharmacological class	Representatives	International common name (DCI)
Atypical antipsychotics/ neuroleptics (second generation)	Aripiprazole	ARIPIPRAZOLUM
	Asenapine	ASENAPINUM
	Cariprazine	CARIPRAZINUM
	Clozapine	CLOZAPINUM
	Loxapine	LOXAPINUM
	Lurasidone	LURASIDONUM
	Olanzapine	OLANZAPINUM
	Quetiapine	QUETIAPINUM
	Paliperidone	PALIPERIDONUM
	Risperidone	RISPERIDONUM
	Sertindole	SERTINDOL
	Tiapride	TIAPRIDUM
	Ziprasidone	ZIPRASIDONUM

CHAPTER 2. ASSESSMENT OF LIPOPHILICITY AND PHARMACOLOGICAL PROPERTIES OF ANTIPSYCHOTIC DRUGS

2.1 LIPOPHILICITY

Lipophilicity reflects the affinity of a molecule for lipid environments and can be used to predict the ability of a drug to cross the blood-brain barrier. For antipsychotic drugs, lipophilicity is an essential condition to facilitate dissolution in lipid cell membranes in order to cross the blood-brain barrier and exert the antipsychotic effect [17,18].

2.1.1 Impact of lipophilicity on drug bioavailability

Assessing the lipophilicity of a molecule serves several key purposes including:

- ✓ explaining the distribution of the molecule in biological systems
- ✓ determining the potential transport routes of pollutants in the environment
- ✓ support for the development process of new medicines
- ✓ selection of the ideal drug composition to optimize both bioactivity and bioavailability [23]

2.2 METHODS FOR DETERMINATION OF LIPOPHILICITY

2.2.1 Direct methods

- *"Shake-flask" method*

This is a traditional technique, the oldest and known direct method, which is based on the partition coefficient of the molecule to be analyzed between n-octanol and water. This method has significant limitations: it is laborious, demanding and time-consuming [25].

2.2.2 Indirect methods

- *Chromatographic methods*

Among the most widely used chromatographic methods are reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase high-performance chromatography (RP-HPLC) [30].

2.3 COMPUTATIONAL METHODS FOR DETERMINATION OF LIPOPHILICITY

While experimental methods for assessing lipophilicity are preferred, computational methods have advantages over experimental methods because they do not require expensive laboratory equipment, reagents, and laborious laboratory work.

These methods emerged to address the demand for fast, simple, high-throughput techniques, aimed at the rapid screening of the investigated compounds. Thus, several techniques have been developed that can be classified into two main groups: substructure-based and property-based [30,31].

2.4 METHODS FOR DETERMINATION OF THE BIOACTIVITY OF ANTIPSYCHOTIC DRUGS AND ACTIVE PRINCIPLES FROM PLANTS

- **Description of the process for determining bioactivity using machine learning techniques**

- The computation of molecular descriptors
- Selection of descriptors. Lipinski's descriptors
- Clasification of models for QSAR
- Evaluation and validation

2.5 ANTIOXIDANT MECHANISM OF ACTION OF DRUGS AND PLANTS WITH ANTIPSYCHOTIC EFFECTS

Another approach in the pathophysiology of schizophrenia is the oxidative stress theory. Studies have shown that oxidative stress can may damage neurons, leading to the development of schizophrenic symptoms.

The possible antioxidant mechanisms of action of antipsychotic drugs are neutralization of free radicals, modulation of antioxidant enzymes and reduction of lipid peroxidation. Olanzapine, aripiprazole and ziprasidone are antipsychotic drugs recognized for their antioxidant mechanism of action [36].

2.6 METHODS OF ANALYSIS FOR EVALUATION OF ANTIPSTCHOTIC EFFECT OF DRUGS AND MEDICINAL PLANTS

In vitro assays are based on HPTLC and HPLC methods for the separation, quantification and assessment of receptor binding affinity of drugs and medicinale plants [42,43].

In vivo methods can be behavioral tests on animals, electrochemical methods, comparative studies [44,45].

2.7 ADRENERGIC DRUGS AND THEIR INTERACTIONS WITH ANTIPSYCHOTIC DRUGS VIA ADRENERGIC RECEPTORS

Adrenergic (adrenomimetic) drugs stimulate the sympathetic nervous system by activating adrenergic receptors, α and β, G protein-coupled receptors. Each type of receptor is classified into specific subtypes:

- ✓ α_1 adrenergic receptors are located in vascular smooth muscles, and their activation produces vasoconstriction and an increase in blood pressure
- ✓ α2 adrenergic receptors are located in presynaptic nerve endings and they inhibit the release of norepinephrine and supress sympathetic activity
- ✓ β1 adrenergic receptors are predominantly located in the heart and their activation leads to an increase of heart rate and the force of cardiac contractions
- ✓ β2 adrenergic receptors are located in the smooth muscles of the bronchi and blood vessels, and their activation leads to bronchodilation and vasodilation [46,47].

Antipsychotic drugs primarily exert their effects by antagonizing dopaminergic and serotonergic receptors, but most antipsychotic drugs also antagonize α-adrenergic receptors, contributing both to the improvement of schizophrenia symptoms and to the occurrence of adverse effects. Adverse effects related to binding to adrenergic receptors are orthostatic tension, weight gain, and metabolic syndrome [7,8,50].

PERSONAL CONTRIBUTIONS

CHAPTER 3. STUDY ON BINDING AFFINITY PROFILE, BIOACTIVITY AND LIPOPHILICITY OF SELECTED ANTIPSYCHOTIC DRUGS [51]

3.1 INTRODUCTION

For selected antipsychotic drugs authorized in Romania [15] (Haloperidol, Risperidone, Aripiprazole, Olanzapine, and Quetiapine) theoretical and experimental lipophilicity parameters were discussed in terms of correlations with topological polar surface area (TPSA), binding affinity profile, data from literature and bioactivity estimated by computational methods.

The aim of this study was to obtain a comprehensive profile regarding the relationship between structure, lipophilicity, and biological activity of antipsychotic drugs most clinically used in Romania.

3.2 EXPERIMENTAL METHODS

3.2.1. Computational methods

Mol inspiration cheminformatics software was used to evaluate the topological polar surface area (TPSA), lipophilicity and bioactivity of the selected antipsychotic drugs [52].

3.2.2 Chromatographic methods

Retention factors, R_f, were determined for all types of chromatographic plates used and lipophilicity parameters were calculated (values of the parameters mR_M, R_{M0}) based on the values of the retention factors obtained for five different fractions of the organic solvent in the composition of the mobile phase using the below equations:

$$R_M = \log(1/R_f - 1) \quad (1)$$

Where $R_{f=}$ compound migration distance / solvent front migration distance

$$R_M = R_{M0} + bC \quad (2)$$

Where R_M is the molar retention, C is the volume fraction of the organic solvent in the mobile phase, b is the slope of the linear regression line (considered an alternative measure of lipophilicity), and R_{M0} is the molar retention obtained by extrapolation for pure water as the mobile phase (mobile phase 100% water)

$$mR_M = \left(\sum_{i=1}^{5} R_{M_i}\right)/5 \quad (3)$$

Where mRM represents the average of individual R_M values [55]

3.3 RESULTS AND DISCUSSIONS

3.3.1 Evaluation of receptor binding affinity profile

Assessing the correlation between the structure and biological activity of antipsychotic drugs helps to understand how the chemical structure influences the interaction of the molecule with different neurotransmitters in the brain, ultimately influencing the drug's efficacy and safety profile.

Receptor binding affinity of antipsychotic drugs refers to the ability of a drug to bind specific receptors in the brain. Different antipsychotic drugs have different affinity for different receptors and this can affect their bioavailability.

Antipsychotic drugs interact with receptors of various neurotransmitters, by binding to G protein-coupled receptors [56] (GPCR-G protein coupled receptors), dopaminergic receptors, subtypes 1, 2, 3, 4, 5-HT2 serotonin receptors, subtypes 2A, 2B, 2C, muscarinic receptors M_1, subtype 1, adrenergic receptors, type 1, α_1, adrenergic receptors type 2, α_2, A, B, C and histamine receptors, H_1, subtype 1.

Receptor binding affinity profile is expressed in Ki values (Nm). Values between 100>ki<1000 indicates weak potency; values between 10>ki<100 indicates moderate potency; values between 1>ki< 10 indicates strong potency; and values 1<ki indicates very strong potency. [53,54].

The structure of antipsychotic drugs influences the affinity for receptor binding, therefore the therapeutic effect and the occurrence of side effects. Typical or first-generation antipsychotic drugs act by strong antagonism at dopamine D_2 receptors, improve the positive symptoms of schizophrenia, but have irreversible extrapyramidal side effects.

Atypical or second-generation antipsychotic drugs exhibit a more intricate mechanism of action. Beyond their antagonist or agonistic effect on dopaminergic receptors, they also act by blocking 5-HT_2 serotonergic receptors. This combined action is the main feature of the mechanism of action of atypical antipsychotic drugs and leads to the improvement of both positive and negative symptoms of schizophrenia and the low incidence of extrapyramidal side effects.

3.3.2 Bioavailability and bioactivity results

Mol inspiration cheminformatics software was used to calculate the bioactivity score of studied antipsychotics against common human receptors such as G protein-coupled receptors (GPCR), ion channel, kinase, nuclear receptor and protease. The bioactivity of an active substance is defined based on the bioactivity score which represents how a compound interacts with specific targets such as enzymes or receptors. A compound is highly active if it has a bioactivity score greater than 0, and an inactive compound has a bioactivity score less than -5.

According to the obtained results antipsychotic drugs have different levels of bioactivity against common human receptors, including G protein coupled receptors (GPCRs), ion channels, kinase, nuclear receptor and protease.

The diverse bioactivity profile of antipsychotic drugs contributes to the therapeutic effect of antipsychotic drugs but may also account for class- specific side effects, including extrapyramidal symptoms, sedation, weight gain, or metabolic side effects.

A strong bioactivity of all antipsychotics on G protein-coupled receptors (GPCRs) was observed (Figure 5).

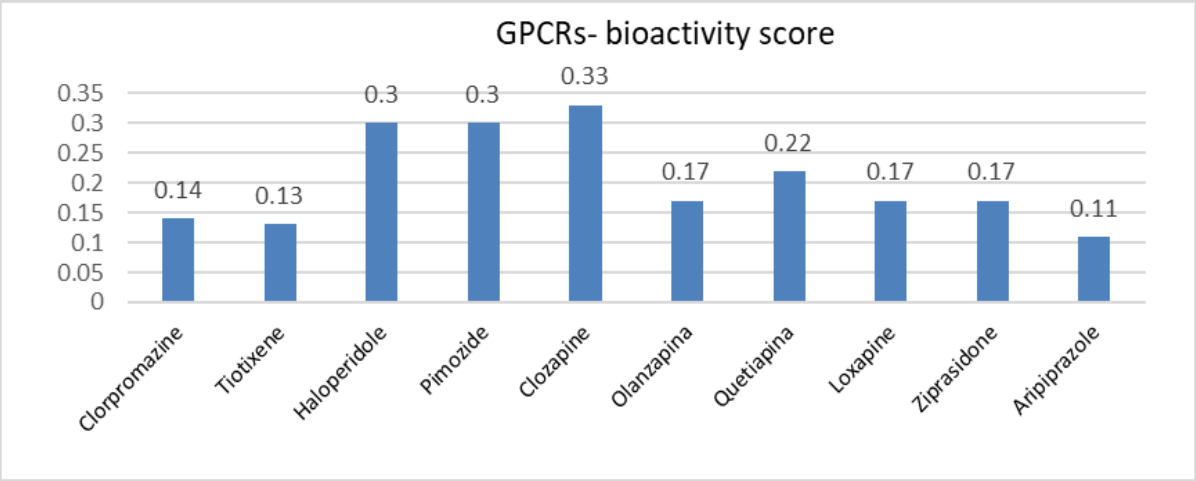

Figura 5. Bioactivity score against G protein-coupled receptors (GPCRs)

The acceptance criteria are represented by determining the bioactivity score parameter. If the bioactivity score is >0 the compound is active, between -5.0 – 0.0 the compound is moderately active, and <-5.0 the compound is inactive.

Evaluating receptor binding affinity for GPCR monoamine, inhibitory or activating effects [67], antipsychotic drugs are GPCR ligands (agonists, antagonists, inverse agonists and partial agonists) [68].

In Figure 5 above, it can be observed that clozapine is the most effective antipsychotic, with the highest bioactivity score.

By comparing the results of the bioactivity score with the receptor binding affinity profile, it can be concluded that clozapine is the most effective antipsychotic, as it exhibits the highest bioactivity score and interacts with a broad spectrum of GPCR receptors: dopaminergic, serotoninergic, muscarinic, adrenergic and histaminergic receptors.

The molecular polar surface area (TPSA), the surface belonging to the polar atoms, is a parameter that has been shown to correlate well with passive molecular transport through membranes and therefore allows the prediction of the transport properties of drugs and is linked to drugs bioavailability [69].

Figure 6 below shows the TPSA values for the investigated antipsychotic drugs. It is observed that olanzapine presents the lowest value, which indicates that this molecule has the best ability to cross the blood-brain barrier, influencing the bioavailability and efficacy of olanzapine.

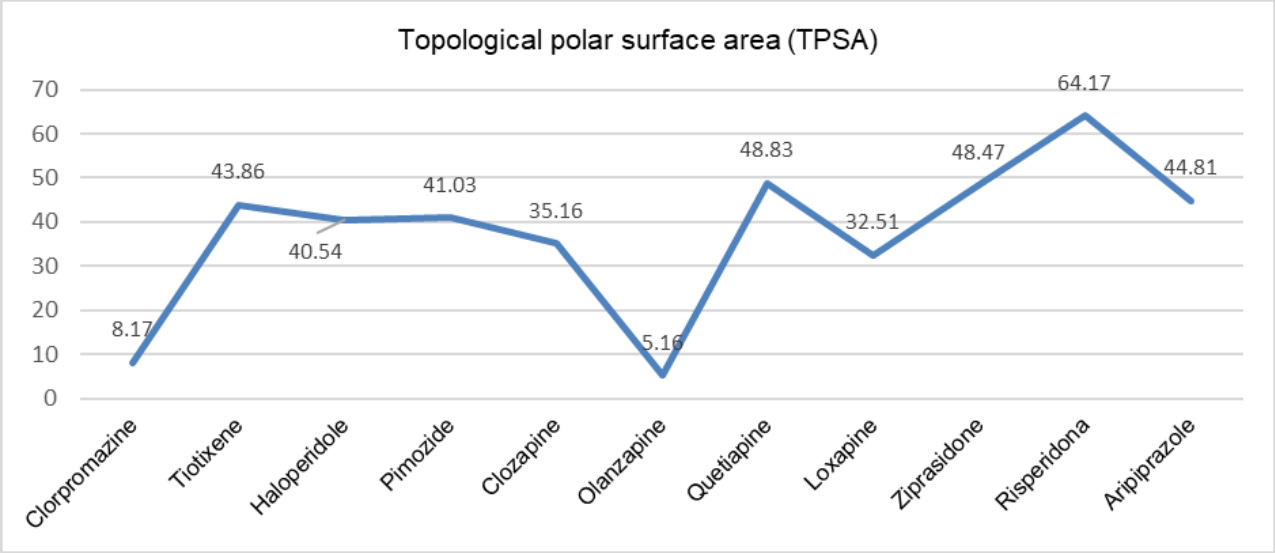

Figura 6. Topological polar surface area evaluation results

3.3.3 Chromatographic lipophilicity parameters

The lipophilicity of selected antipsychotic drugs was determined from retention factors using chromatographic plates with different polarity.

The lipophilicity variation profile (RM parameters, Figure 7) of the selected drugs haloperidole, risperidone, aripiprazole, olanzapine and quetiapine revealed a linear increasing of lipophilicity with increase of organic component fraction in mobile phase composition.

This trend is important for understanding the pharmacokinetic behavior of drugs, as lipophilicity influences absorption, distribution, metabolism, excretion. Lipophilic drugs cross the blood-brain barrier which allows them to exert their therapeutic effect, therefore this condition is essential for antipsychotic drugs.

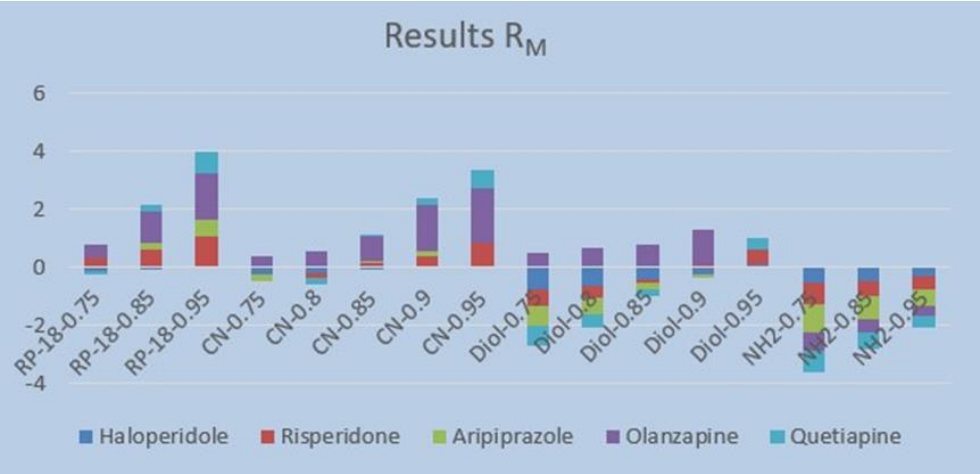

Figura 7. Profile of lipophilicity (RM) parameters for the investigated drugs on different chromatographic plates (RP-18, CN, Diol and NH2 modified silica gel plates) using different acetonitrile fraction in mobile phase composition

Profile for the R_M parameters obtained for selected drugs on the investigated chromatographic plates are presented in Figure 8. It can be observed that the investigated antipsychotics have a linear lipophilicity profile in all cases, the lipophilicity being affected by the interaction of drugs with stationary phase.

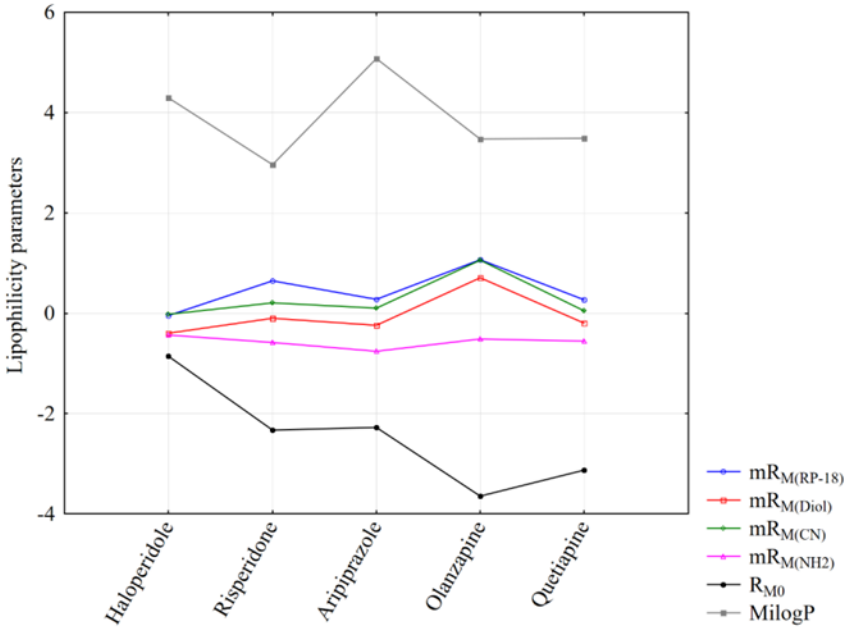

Figure 8. Correlations between experimental and computed lipophilicity parameters for the investigated drugs.

In the conducted analysis Milog P lipophilicity parameters were calculated and a correlation study was developed based on the lipophilicy experimental and computationally determined and bioavailability parameters for the investigated drugs. The results of these correlations are presented in table 8.

Tabel 8. The correlation between lipophilicity and different computed bioavailability parameters for selected antipsychotic drugs (haloperidole, risperidone, aripiprazole, olanzapine and quetiapine). Bold values show a statistically significant correlation.

Bioavailability parameters	mR_M				R_{M0}				MilogP	TPSA	GPCR	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
	RP-18	DIOL	CN	NH_2	RP-18	DIOL	CN	NH_2								
$mR_{M(RP-18)}$	1.00	**0.93**	**0.91**	-0.03	**-0.78**	0.31	**-0.86**	-0.24	-0.55	-0.27	-0.03	-0.60	0.59	**-0.96**	**-0.89**	-0.31
$mR_{M(Diol)}$	**0.93**	1.00	**0.99**	0.13	**-0.76**	0.57	**-0.75**	-0.04	-0.40	-0.52	-0.25	-0.30	**0.76**	**-0.96**	**-0.94**	-0.21
$mR_{M(CN)}$	**0.91**	**0.99**	1.00	0.16	-0.69	0.64	-0.68	-0.01	-0.35	-0.47	-0.26	-0.28	**0.71**	**-0.96**	**-0.95**	-0.15
$mR_{M(NH2)}$	-0.03	0.13	0.16	1.00	0.21	0.02	-0.12	**0.97**	-0.47	-0.27	0.43	0.59	0.23	-0.06	0.12	**0.83**
$R_{M0(RP-18)}$	**-0.78**	**-0.76**	-0.69	0.21	1.00	-0.15	0.84	0.36	0.43	0.64	0.36	0.35	**-0.83**	0.64	0.66	0.69
$R_{M0(DIOL)}$	0.31	0.57	0.64	0.02	-0.15	1.00	0.12	0.02	0.46	-0.37	-0.68	0.15	0.36	-0.52	-0.70	0.00
$R_{M0(CN)}$	**-0.86**	**-0.75**	-0.68	-0.12	0.84	0.12	1.00	0.08	**0.84**	0.38	-0.19	0.45	-0.67	0.71	0.56	0.31
$R_{M0(NH2)}$	-0.24	-0.04	-0.01	**0.97**	0.36	0.02	0.08	1.00	-0.29	-0.26	0.35	**0.74**	0.14	0.14	0.28	**0.86**
MilogP	-0.55	-0.40	-0.35	-0.47	0.43	0.46	**0.84**	-0.29	1.00	0.12	-0.65	0.26	-0.36	0.41	0.14	-0.15
TPSA	-0.27	-0.52	-0.47	-0.27	0.64	-0.37	0.38	-0.26	0.12	1.00	0.58	-0.49	**-0.93**	0.24	0.33	0.24
GPCR	-0.03	-0.25	-0.26	0.43	0.36	-0.68	-0.19	0.35	-0.65	0.58	1.00	-0.22	-0.45	0.09	0.38	0.57
Ion channel modulator	-0.60	-0.30	-0.28	0.59	0.35	0.15	0.45	0.74	0.26	-0.49	-0.22	1.00	0.18	0.50	0.44	0.47
Kinase inhibitor	0.59	**0.76**	0.71	0.23	**-0.83**	0.36	-0.67	0.14	-0.36	**-0.93**	-0.45	0.18	1.00	-0.55	-0.58	-0.32
Nuclear receptor ligand	**-0.96**	**-0.96**	**-0.96**	-0.06	0.64	-0.52	**0.71**	0.14	0.41	0.24	0.09	0.50	-0.55	1.00	**0.95**	0.16
Protease inhibitor	**-0.89**	**-0.94**	**-0.95**	0.12	0.66	**-0.70**	0.56	0.28	0.14	0.33	0.38	0.44	-0.58	**0.95**	1.00	0.32
Enzyme inhibitor	-0.31	-0.21	-0.15	0.83	0.69	0.00	0.31	**0.86**	-0.15	0.24	0.57	0.47	-0.32	0.16	0.32	1.00

3.4 CONCLUSIONS

Dopamine and serotonin receptors are the main GPCRs targeted by antipsychotic drugs. Clozapine exerts the strongest bioactivity of all antipsychotics on G protein-coupled receptors (GPCRs).

Among the investigated bioavailability parameters polar surface area (TPSA) is strongly correlated with kinase inhibitor activity of antipsychotic drugs, while protease inhibitor and nuclear receptor ligand parameters are directly correlated for such kinds of drugs.

A statistically significant correlation is revealed between the experimental lipophilicity parameters mR_M obtained on RP-18, Diol, and CN modified stationary phases. Based on the significant correlation of these experimental parameters with nuclear receptor ligand and protease inhibitor parameters, respectively, the lipophilicity estimated on different stationary phases are relevant for the prediction of antipsychotic action of such kind of drugs.

Also, the enzyme inhibitory activity of antipsychotics can be estimated by experimental lipophilicity parameters mR_M and R_{M0} determined on NH_2 stationary phases. Based on the obtained correlation results it can be concluded that lipophilicity estimated on different stationary phases is a useful parameter for bioactivity prediction in the case of antipsychotics drugs.

CHAPTER 4. IDENTIFICATION AND EVALUATION OF THE ANTIPSYCHOTIC EFFECT OF THE ACTIVE PRINCIPLES FROM PLANTS

There is an increased trend of scientific research into the active principles of plants for the treatment of mental disorders. Among these mental disorders, schizophrenia is a topic of major interest driven by the need for more effective treatments with minimized side effects compared to current antipsychotic drugs and improved patient adherence to treatment.

Medicinal plants have been used throughout history in traditional medicine, due to their therapeutic properties. Plant extracts are widely utilized in traditional medicine, either independently or in combination with drugs, but the biological activity of these plant extracts has been evaluated for only a limited number of these extracts. In addition to the therapeutic benefits, natural extracts, like other treatment options, may also cause side effects that need to be assessed and taken into consideration [34].

This chapter aims to assess the antipsychotic effects of active principles from plants through several stages, as outlined below:

- Conducting database research to identify plants with antipsychotic effects

- Compiling data in tabular form that includes details such as the species, family, vegetative organ utilized, active principles from plants responsible for antipsychotic effect and the chemical class to which these identified active principle belongs
- Classification of the identified active principles with antipsychotic effects
- Determining the bioactivity of the active principles identified based on their chemical structure and using the molinspiration cheminformatics software application
- Evaluation of the results
- Evaluating the similarity between the biological activity of antipsychotic drugs and active plant principles with antipsychotic effects.

The purpose of this study is to obtain an overview of medicinal plants with antipsychotic effects, the chemical classes of active principles most important for antipsychotic activity, to determine the bioactivity score and to perform an evaluation of the active principles with the highest bioactivity score.

From the active principles with the highest bioactivity score, those available in Romania will be selected. Their correlations with the biological activity results of the antipsychotic drugs will be evaluated to identify potential compounds with antipsychotic effects. The objective is to identify active principles with therapeutic potential that could be associated with antipsychotic drug treatment.

4.1 DENTIFICATION OF ACTIVE PRINCIPLES FROM PLANTS WITH ANTIPSYCHOTIC EFFECTS

To determine the active principles from plants with antipsychotic effects, a literature review was conducted to identify such plants and their respective active principles responsible for the therapeutic effect.

A total of 78 active principles with antipsychotic effects have been identified, the most common being quercetin and rutin. The high frequency of quercetin and rutin in the study of medicinal plants with antipsychotic effects underlines the importance of these active principles in the treatment of mental disorders.

Most of the identified active principles with antipsychotic effect belongs to the chemical classes of flavonoids, alkaloids, terpenes, lignans and phenolic acids. These findings emphasize the essential role of these chemical classes in the study of antipsychotic activity of medicinal plants.

4.2 DETERMINATION OF BIOACTIVITY OF ACTIVE PRINCIPLES FROM PLANTS

The bioactivity of the active principles was determined using the same method applied as in the evaluation of antipsychotic drugs. The previous chapter highlighted the significant impact that the variety of chemical structure of antipsychotic drugs has in their interactions with neurotransmitter receptors and their therapeutic effect.

Based on the chemical structure of the active principle and with the molinspiration cheminformatics software application, the bioactivity against common human receptors, such as: GPCR ligand, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and enzyme inhibitors were calculated.

The bioactivity of 38 active principles was calculated, with the results presented in table 12, below:

Table 12. Result of bioactivity for selected active principles

No.	Active principles	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	bufanidrine [74]	0.36	0.08	-0.27	-0.13	-0.15	0.32
2	bufanamine [74]	0.49	0.28	-0.31	-0.08	0.06	0.33
3	quercetin [74,75]	-0.06	-0.19	0.28	0.36	-0.25	0.28
4	rutin [95, 103]	-0.05	-0.52	-0.14	-0.23	-0.07	0.12
5	reserpine [74,77]	0.1	-0.36	-0.39	-0.36	-0.02	-0.21
6	yohimbin [74,77]	0.47	0.36	-0.14	-0.03	0.11	0.16
7	scopoletine [131]	-1	-0.65	-0.95	-0.81	-1.16	-0.24
8	xysmalorine [74]	-0.43	-1.38	-1.2	-0.72	-0.35	-0.24
9	uzarine [74]	-0.38	-1.17	-1.08	-0.79	-0.28	-0.23
10	bergenine [74,78]	0.06	-0.09	-0.09	-0.08	-0.14	0.35
11	gallic acid [139]	-0.77	-0.26	-0.88	-0.52	-0.94	-0.17
12	linalool [74,82]	-0.73	0.07	-1.26	-0.06	-0.94	0.07
13	isorhamnetin [95, 107, 108]	-0.11	-0.27	0.21	0.27	-0.27	0.2
14	(S)-naringenin [74,85,86]	0.03	-0.2	-0.26	0.42	-0.12	0.21
15	piperine [137,138]	0.15	-0.18	-0.13	-0.13	-0.1	0.04

No.	Active principles	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
16	scopolamine [74,91]	0.58	0.23	0.06	0.11	0.28	0.35
17	β−asarone [95, 96]	-0.71	-0.43	-0.72	-0.47	-0.97	-0.39
18	egeline [95,98]	0.21	0.24	-0.25	-0.18	-0.09	0.11
19	marmeline [95,98]	0.16	-0.14	-0.28	0.15	-0.05	0.13
20	Δ9-tetrahydrocannabinol [95, 105, 106]	0.61	-0.03	-0.31	0.6	-0.04	0.51
21	cannabidiol [95, 105, 106]	0.35	-0.14	-0.48	0.38	-0.19	0.33
22	bilobalide [121]	0.75	0.11	-0.23	0.35	0.45	0.23
23	ginkgolide [121]	1.15	0.07	-0.29	0.14	0.34	0.35
24	hypericine [124, 125]	-0.01	-0.23	0.1	0.19	-0.08	0.1
25	crysin [153]	-0.11	-0.08	0.15	0.3	-0.3	0.26
26	salidroside [140,141]	0.35	0.29	0.13	0.06	0.19	0.59
27	catechin [142]	0.41	0.14	0.09	0.6	0.26	0.47
28	berberine [145,146]	-0.11	0.71	-0.27	-0.78	-0.35	0.82
29	magnoflorine [145,146]	0.37	0.78	-0.2	-0.26	-0.19	0.26
30	valerenol [147,148]	-0.03	0.04	-0.89	0.57	-0.37	0.44
31	withanolide [150,151]	0.05	0.3	-0.5	0.73	0.16	1.07
32	jujubozide [152]	-3.65	-3.76	-3.81	-3.73	-3.59	-3.6
33	spinosin [152]	0.04	-0.45	-0.11	-0.13	-0.01	0.19
34	isoliquiritin [154]	0.03	-0.32	-0.39	0.32	-0.21	0.17
35	glycyrrhizin [154]	-1.78	-3.09	-3.09	-2.36	-1.26	-1.9
36	apigenin [74,84]	-0.07	-0.09	0.18	0.34	-0.25	0.26
37	carnosic acid [158]	0.41	0.24	-0.24	0.72	-0.01	0.32
38	rosmarinic acid [158]	0.17	-0.08	-0.18	0.57	0.15	0.24

To assess the antipsychotic potential of the active principles, it is important to analyze the bioactivity determination results, specifically the bioactivity scores calculated for the most significant receptors and ligands.

- **G protein-coupled receptors:** antipsychotic drugs are ligands of GPCR receptors acting as agonists, antagonists, inverse agonists or partial agonists [160]. Analyzing the bioactivity score, reveals that all investigated compounds exhibit moderate active or highly active activity against G protein-coupled receptors.
- **Ion channel modulators**: voltage dependent ion channels are involved in the pathophysiology of schizophrenia. Some antipsychotic drugs act as direct inhibitors of these voltage-gated ion channels [161, 162].
- **Enzyme inhibitors**: such as cytochrome P450 inhibitors influence the metabolism process of antipsychotic drugs. Knowing the main metabolic pathways of antipsychotic drugs can lead to increased therapeutic effect and decreased adverse effects [163].
- Analyzing the bioactivity score of the investigated active principles indicates that all these compounds demonstrate moderate to high activity against all types of ligands and receptors examined.

4.3 ASSESSMENT OF THE ANTIPSYCHOTIC EFFECT OF THE ACTIVE PRINCIPLES

In line with the analysis of the antipsychotic drugs bioactivity against GPCR ligands, which are regarded as the the main target for most antipsychotic drugs, the bioactivity of the active principles against GPCR receptors is graphically represented in Figure 10.

Based on the bioactivity scores, all active principles with antipsychotic effects exhibit either moderate or high activity, as outlined below:

- ✓ 18 moderately active compounds, bioactivity score values between -3.65 and 0.00
- ✓ 22 highly active compounds, with bioactivity score values greater than 0.00

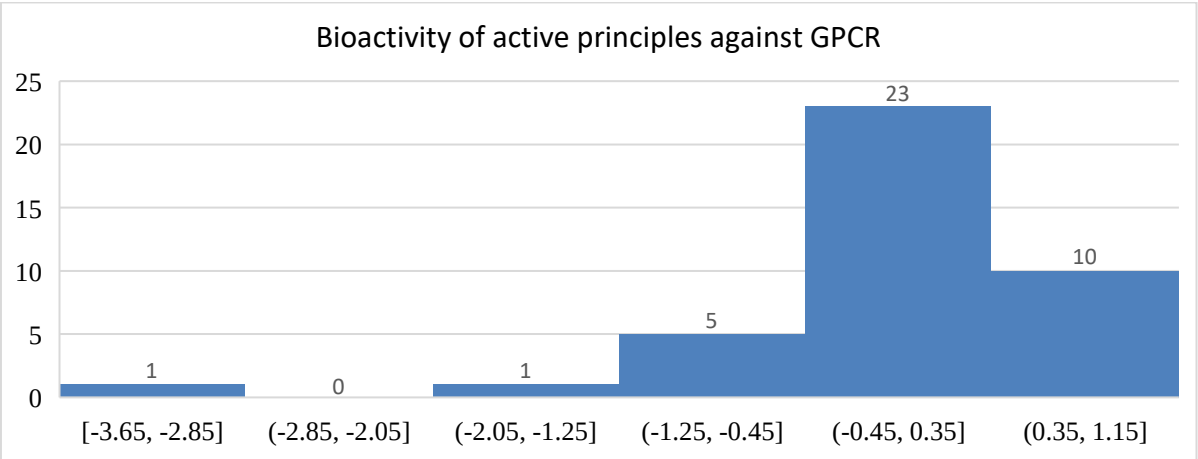

Figure 10. Bioactivity of active principles against GPCR

The acceptance criteria are represented by determining the bioactivity score parameter. If the bioactivity score is >0 the compound is active, between -5.0 – 0.0 the compound is moderately active and <-5.0 the compound is inactive.

From the active principles with identified antipsychotic effects, those with the highest bioactivity scores were selected, 12 compounds with scores ranging from 0.17 to 1.15, as illustrated in Figure 11. The evaluation will focus on the compounds with the highest scores and derived from plants available in Romania.

In Figure 11 - Bioactivity evaluation of highly active compounds, are represented the top 12 compounds with the highest bioactivity score against GPCR receptors.

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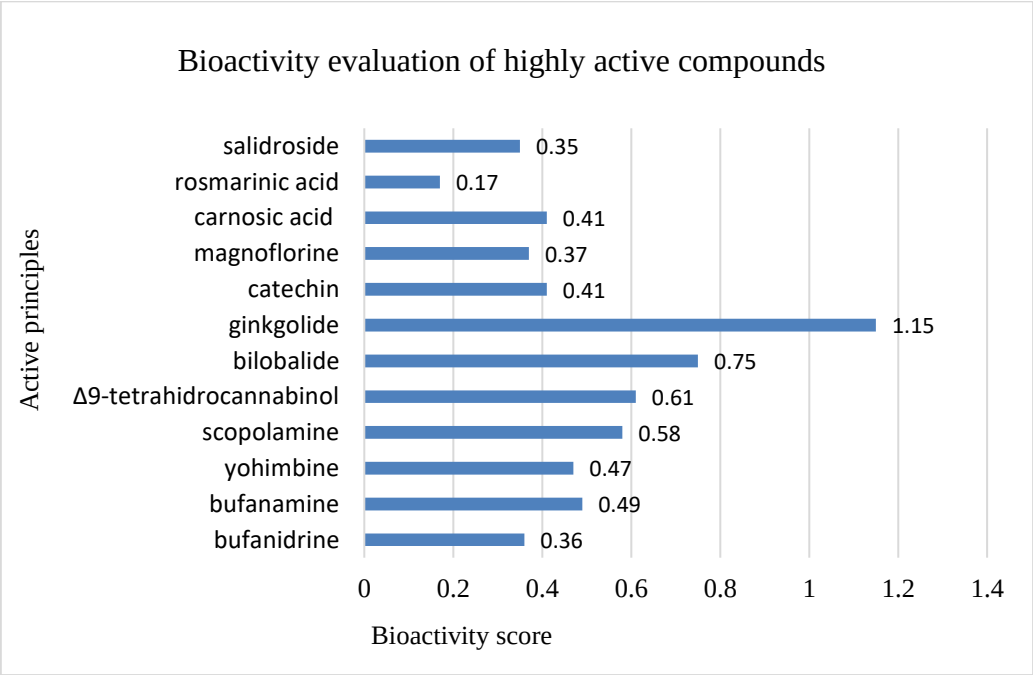

Figura 11. Bioactivity evaluation of highly active compounds

The acceptance criteria is represented by determining the bioactivity score parameter. If the bioactivity score is >0 the compound is active, between -5.0 – 0.0 the compound is moderately active and <-5.0 the compound is inactive.

Analyzing the 12 highly active compounds, with the highest bioactivity score, it was determined that the most promising medicinal plants with antipsychotic effects, available in Romania are *Ginkgo biloba* and *Rosmarinus officinalis*.

Ginkgo Biloba is known for its antioxidant and neuroprotective properties [121], while *Rosmarinus officinalis* is particularly valued for its anti-inflammatory properties [158].

4.4 ANALYSIS OF THE SIMILARITY BETWEEN THE BIOLOGICAL ACTIVITY OF ANTIPSYCHOTIC DRUGS AND THE ACTIVE PRINCIPLES

In previous chapters the receptor binding affinity profile for selected antipsychotic drugs has been evaluated.The relationship between the structure and antipsychotic activity of these drugs against G protein-coupled receptors (GPCRs) was studied. Antipsychotic drugs are GPCR receptor ligands acting as agonists, antagonists, inverse agonists or partial agonists.

Molinspiration cheminformatics software application was used to calculate the bioactivity score of antipsychotic drugs and active principles from plants against GPCR receptors.

The active principles with antipsychotic effects that exhibits the highest activity score were selected and focusing on those present in plants available in Romania, specifically *Ginkgo biloba* and *Rosmarinus officinalis*.

Figure 12 below shows the bioactivity score for the investigated antipsychotic drugs and the active principles that exhibits the highest bioactivity score and are found in plants also available in Romania.

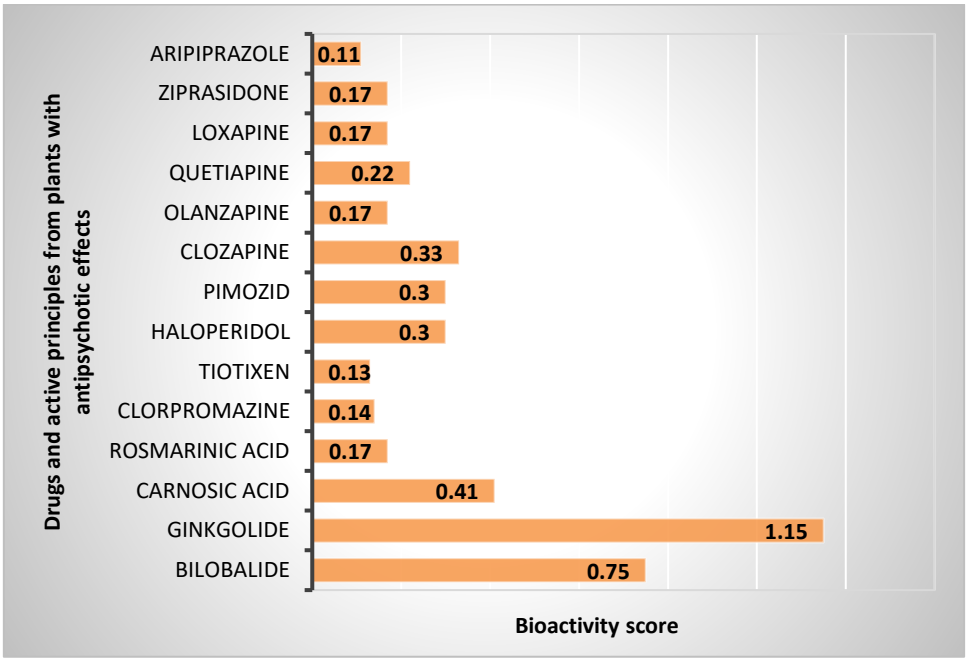

Figure 12. Evaluation of the bioactivity of antipsychotic drugs and active principles

These active principles from plants demonstrate a higher bioactivity compared to the investigated antipsychotic drugs authorized in Romania, indicating their potential for inclusion in antipsychotic treatments.

CHAPTER 5. NEW METHODS FOR THE RAPID AND SIMULTANEOUS DETERMINATION OF ADRENERGIC DRUGS PROHIBITED IN SPORTS [164]

5.1 INTRODUCTION

The aim of this work is development of a protocol for rapid detection and determination of some adrenergic drugs prohibited for consumption in athletes, using thin layer chromatography coupled with different detection modes and image processing analysis.

5.2 MATERIALS ȘI METODE

5.2.2 Chromatographic conditions

The separation of fenoterol, procaterol, clenbuterol, terbutaline and isoprenaline was performed using High-performance HPTLC Silica gel 60 F_{254S} (20 cm x 10 cm) chromatographic plates and mixtures of isopropyl alcohol, ethyl acetate and ammonia in various proportions (10:10:5 v/v/v/; 10:20:5 v/v/v/; 20:10:5; 20:25:5 v/v/v and 20:25:1.5 v/v/v) as mobile phases. In each case, before the plate development procedure, the chromatographic chamber was saturated with vapors of solvents mixture for 15 minutes.

5.2.4 Validation of the HPTLC-IA method

The proposed method was validated by linearity range, accuracy, precision, limit of detection, limit of quantification according to the ICH guidelines (ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology, Q2(R1), ICH, Geneva, Switzerland, 2005, http://www.ich.org). Method specificity was evaluated for determination of fenoterol, procaterol, clenbuterol, terbutaline and isoprenaline drugs in urine samples.

5.3 RESULTS AND DISCUSSIONS

5.3.1 Development and optimization of HPTLC separation

Various parameters such as mobile phase composition, detection modes and color scale selection for image processing were investigated in order to obtain satisfactory results for simultaneous analysis of these drugs.

For mobile phase composition, eco-friendly solvents were selected according to green philosophy (reuse, replace, reduce) of GlaxoSmithKline (GSK) solvent sustainability guide. Mixtures of isopropyl alcohol, ethyl acetate and ammonia in variable ratios (10:10:5 v/v/v/;

10:20:5 v/v/v/; 20:10:5; 20:25:5 v/v/v and 20:25:1.5 v/v/v) were tested for efficient separation of the selected drugs.

Some of the preliminary trials of isopropyl alcohol and ethyl acetate mixtures revealed the promising capability to separate the analyzed drugs in form of streaks rather than compact spots.

Considering the basic groups from the structure of analyzed drugs, the tailings of the chromatographic spots were resolved by addition of ammonia.

Isopropyl alcohol - ethyl acetate - ammonia (25%) in the proportion of 20: 25: 1.5 (v/v/v) conducted to compact bands without tailings and optimum separation of the analyzed drugs with satisfactory retention factor values: R_f (fenoterol) = 0.61±0.03; R_f (procaterol) = 0.29±0.04; R_f (clenbuterol) = 0.40±0.02; R_f (terbutaline) = 0.16±0.01; R_f (isoprenaline) = 0.09±0.03.

5.3.2 Selection of the parameters for HPTLC analysis

For detection procedure, chromatographic plate was first documented under UV light at 254nm and 366nm. The acquired images were subsequently opened in ImageDecipher-TLC software and color intensities of the spots were converted into analytical signals in form of the peak area using the green, red, blue and grey scale selection. In these conditions only clenbuterol and procaterol were detected (Figure 13). In both cases, the highest values for the spot area were obtained by green scale selection.

Using the DPPH• and respectively ABTS+• radical solution as detection reagents, four of the analyzed drugs (fenoterol, procaterol, terbutaline and isoprenaline) were detected.

In this approach developed plates are immersed in radical solutions and compounds with radical scavenging activity appear as yellowish-white spots produced by bleaching the purple color of the DPPH• reagent and as white spots by bleaching the green color of the ABTS+•. Interesting to note that fenoterol and terbutaline drugs undergo oxidative degradation with ABTS•+ leading to intense, yellow-colored compounds. Contrast variation, invert procedure and different color scale selection (red, green, blue and grey) were applied further for processing of the RGB image of chromatographic plate and quantification of the separated drugs (Figure 13).

5.3.3 Validation of the developed HPTLC- IA method

For all the analyzed drugs, a good linear dependence was observed between the spot area and drug concentration (coefficient of determination higher than 0.9982. R^2 = 0.9996 for fenoterol, R^2 =0.9988 for procaterol, R^2 = 0.9992 for clenbuterol R^2 = 0.9982 for terbutaline, R^2 = 0.9997 for isoprenaline).

In the selected chromatographic conditions, fenoterol presented a linear range between 0.20− 1.80 µg/ spot, procaterol from 0.40 to 1.60 µg/spot, clenbuterol from 0.40 to 2.50 µg/spot, terbutaline from 0.40 to 1.80 µg/spot and isoprenaline from 0.08−1.60 µg/spot).

The use of ABTS+• reagent and red scale selection conducted to the most sensible detection (lower limit of detection, LOD) of fenoterol (LOD = 0.07±0.03 µg/spot), procaterol (LOD = 0.09± 0.03 µg/spot) and terbutaline (LOD = 0.10 ± 0.04 µg/ spot) while the use of DPPH• with green scale selection revealed the most sensible detection mode for isoprenaline (LOD = 0.06± 0.02 µg/spot). The best results for clenbuterol (detected only in UV 254 nm) were obtained by green scale selection (LOD = 0.22± 0.06 µg/spot).

The accuracy of the method was evaluated by determined amounts of drugs from standard mixtures prepared at three levels of concentration for each of the analyzed drugs (0.50 µg/spot; 1.00 µg/spot; 1.50 µg/spot). Good recovery parameters between 92.16% and 104.10% were obtained for the separated drugs in the analyzed mixtures.

The results for repeatability and intermediate, expressed as the coefficients of variation (CV, %) of the peak area in each case were between 0.62% and 1.46%, indicating a good precision of the method in the selected chromatographic conditions (less than 3% that are accepted by ICH guidelines methodology).

The specificity of the method was determined in relation to the interferences from other compounds in the urine sample. The method specificity for fenoterol, procaterol, clenbuterol and terbutaline was confirmed by a good resolution of separation and no interferences from urine constituents. For isoprenaline, interference compounds from urine were revealed in both modes of detection using DPPH• and ABTS+• radical respectively.

Due to the retention value of the unidentified interfering compounds, we were unable to quantify isoprenaline in spiked urine samples, so the accuracy of the method was evaluated for fenoterol, procaterol, clenbuterol and terbutaline by analysis of spiked urine samples at two levels of concentration (0.50 µg/spot and 1.00 µg/spot).

The matrix effect study and recovery test of the developed method on real samples analysis was performed by adding appropriate standard mixtures of fenoterol, procaterol, clenbuterol and terbutaline in urine samples.

The matrix effect was investigated by comparing the spot area of the fortified urine sample with the spot area of standard solution at the same concentration using the above-mentioned two concentration levels for six replicate spots for every of the concentration.

The results revealed that the matrix effect in urine is negligible after two times dilution of urine sample since the RSD values were less than 5% in all cases. Recovery results were between

93% and 108% indicating a good accuracy of the developed method for determination of fenoterol, procaterol, clenbuterol and terbutaline in urine.

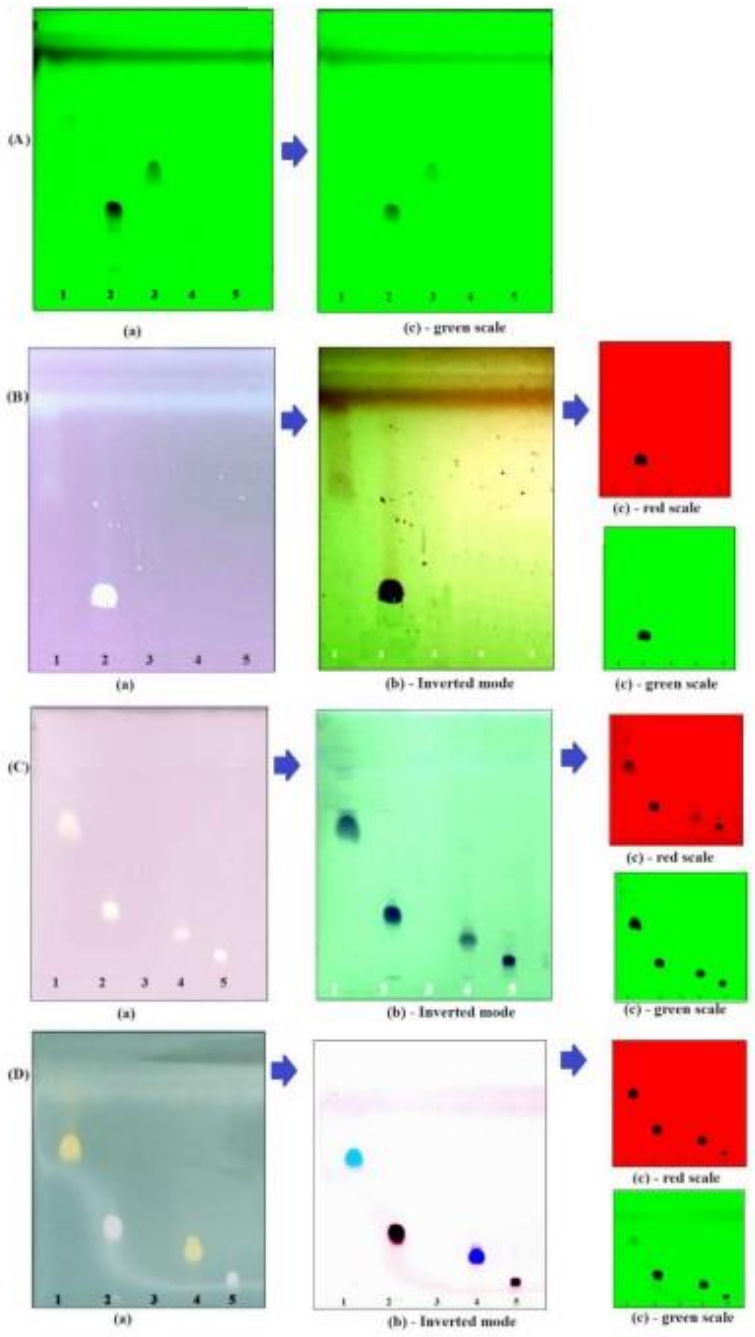

Figura 13. The image of chromatographic plates after separation of the analyzed drugs using different detection modes and different color scale selection for image processing: (A) - UV 254nm; (B) - UV 366nm; (C) - Immersion in DPPH• solution; (D) - Immersion in ABTS+• solution; (a) - RGB image; (b) – inverted mode image (c) – color scale for image processing (1- fenoterol; 2 – procaterol; 3 – clenbuterol; 4 – terbutaline; 5 – isoprenaline).

5.4 CONCLUSIONS

A new and eco-friendly HPTLC method that combine multiple detection modes with image analysis and different color scales selection was proposed for simultaneous determination of fenoterol, procaterol, clenbuterol, terbutaline and isoprenaline drugs. Good separation of the analyzed drugs was obtained on HPTLC Silica gel 60F254S plates using mixture of isopropyl alcohol, ethyl acetate and ammonia 20:25:1.5 (v/v/v) as mobile phase.

With the exception of clenbuterol (that was detected only in UV mode under 254nm), the analyzed drugs presented a sensible detection after reaction with both DPPH• and ABTS+• reagents respectively. The method performance parameters shown that the developed method is accurate and precise for quantification of all of the analyzed drugs.

The use of ABTS+• reagent in combination with the red scale selection for image processing provided the lowest sensibility of detection for fenoterol (LOD = 0.07±0.03 µg/spot), procaterol (LOD = 0.09±0.03 µg/spot) and terbutaline (LOD = 0.10±0.04 µg/spot) while the use of DPPH• and green scale selection revealed the most sensible detection of isoprenaline (LOD = 0.06±0.02 µg/spot).

The specificity and good accuracy of the developed method was also demonstrated for fenoterol, procaterol, clenbuterol and terbutaline drugs in urine samples.

With its ability to provide reliable and accurate results, this method is poised to become an integral part of drug testing and analysis in the doping area.

GENERAL CONCLUSIONS

Antipsychotic drugs exert their bioactivity by modulating the activity of specific brain receptor, primarily dopamine and serotonin receptors. Typical antipsychotics primarily block dopamine D_2 receptors. Atypical antipsychotics are more selective, targeting multiple receptors including both dopamine D_2 and serotonin receptors.

Receptor binding affinity profiles of typical and atypical drugs are correlated with the efficacy and side effects of these drugs. Dopamine and serotonin receptors are the main GPCRs targeted by antipsychotic drugs. Clozapine exerts the strongest bioactivity of all antipsychotics on G protein-coupled receptors (GPCRs).

Among the investigated bioavailability parameters polar surface area (TPSA) is strongly correlated with kinase inhibitor activity of antipsychotic drugs, while protease inhibitor and nuclear receptor ligand parameters are directly correlated for such kinds of drugs.

A statistically significant correlation is revealed between the experimental lipophilicity parameters mR_M obtained on RP-18, Diol, and CN modified stationary phases. Based on the significant correlation of these experimental parameters with nuclear receptor ligand and protease inhibitor parameters, respectively, the lipophilicity estimated on different stationary phases are relevant for the prediction of antipsychotic action of drugs.

Also, the enzyme inhibitory activity of antipsychotics can be estimated by experimental lipophilicity parameters mR_M and R_{M0} determined on NH_2 stationary phases. Based on the obtained correlation results it can be concluded that lipophilicity estimated on different stationary phases is a useful parameter for bioactivity prediction in the case of antipsychotics.

A total of 78 active principles with antipsychotic effects have been identified, the most common being quercetin and rutin. Most of the identified active principles with antipsychotic effect belongs to the chemical classes of flavonoids, alkaloids, terpenes, lignans and phenolic acids.

Among the active principles with antipsychotic effects, high bioactivity score and found in plants available in Romania, two medicinal plants were identified, specifically *Ginkgo biloba* and *Rosmarinus officinalis.*

These active principles from plants demonstrate a higher bioactivity compared to the investigated antipsychotic drugs authorized in Romania, indicating their potential for inclusion in antipsychotic treatments.

Considering the literature on the mechanisms of antipsychotic action of *Ginkgo Biloba*, *Rozmarinus officialis* alongside with the calculated bioactivity of the active principles with antipsychotic effects, which were evaluated against the receptor binding profile and biological activity of selected antipsychotic drugs, it can be concluded that these active principles show promising potential for use in the treatment of schizophrenia.

Ginkgo Biloba, through its active principles, bilobalide, ginkgolide, exhibits antioxidant effects, through neuroprotective activity and which supplements the antipsychotic effect by modulating dopaminergic receptors and correlated with the high bioactivity score, has therapeutic potential both as an adjunctive treatment associated with antipsychotic drugs or as monotherapy to alleviate the symptoms of schizophrenia.

Rozmarinus officialis through the active principles with antipsychotic effects, carnosic acid and rosmarinic acid, due to the neuroprotective, psychostimulant, antidepressant effects and the high bioactivity score, suggests that rosemary can be used as an adjunct treatment to antipsychotic drugs to alleviate the symptoms of schizophrenia.

The results obtained from the study of active principles with antipsychotic action will serve as useful tools for further research to analyze of the medicinal plants *Ginkgo Biloba, Rozmarinus officialis* and antipsychotic drugs.

A new and eco-friendly HPTLC method that combine multiple detection modes with image analysis and different color scales selection was proposed for simultaneous determination of fenoterol, procaterol, clenbuterol, terbutaline and isoprenaline drugs.

Good separation of the analyzed drugs was obtained on HPTLC Silica gel 60F254S plates using mixture of isopropyl alcohol, ethyl acetate and ammonia 20:25:1.5 (v/v/v) as mobile phase. With the exception of clenbuterol (that was detected only in UV mode under 254nm), the analyzed drugs presented a sensible detection after reaction with both DPPH• and ABTS+• reagents respectively. The method performance parameters shown that the developed method is accurate and precise for quantification of all the analyzed drugs.

The use of ABTS+• reagent in combination with the red scale selection for image processing provided the lowest sensibility of detection for fenoterol (LOD = 0.07±0.03 µg/spot), procaterol (LOD = 0.09±0.03 µg/spot) and terbutaline (LOD = 0.10±0.04 µg/spot) while the use of DPPH• and green scale selection revealed the most sensible detection of isoprenaline (LOD = 0.06±0.02 µg/spot). The specificity and good accuracy of the developed method was also demonstrated for fenoterol, procaterol, clenbuterol and terbutaline drugs in urine samples.

With its ability to provide reliable and accurate results, this method is poised to become an integral part of drug testing and analysis in the doping area.

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2) **Melinda Szasz,** Dorina Casoni, Claudia Cimpoiu, Similarity of structure and biological activity relationship between antipsychotic drugs and plants with antipsychotic effect, 4th Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE IV), Debrecen, Hungary, 1-3 June 2023, oral presentation.

3) Dorina Casoni, **Melinda Szasz**, Ileana-Maria, Sensitive HPTLC Method for simultaneous determination of adrenergic drugs prohibited in sports, 27th International Symposium on Separation Sciences, Cluj-Napoca, Romania, 24-27 September 2023, oral presentation.