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“BABEŞ-BOLYAI” UNIVERSITY CLUJ-NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING
ORGANIC CHEMISTRY DEPARTMENT

Sanda – Rodica Radu (Bota)

CONTRIBUTION TO CINCHONA ALKALOIDS CHEMISTRY

Ph.D.Thesis Abstract

Scientific Adviser:
Prof. dr. Luminiţa Silaghi-Dumitrescu

Cluj-Napoca 2012

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Keywords: *cinchona alkaloids, nucleophilic substitution , azabiciclo [3.2.2] nonan, Swern oxidation, condensation with nitrogen compounds, esters of QCI, QCD, amides of truncated alkaloids, oxidation, separation of enantiomers, HPLCvanalysis, HPLC method validation*

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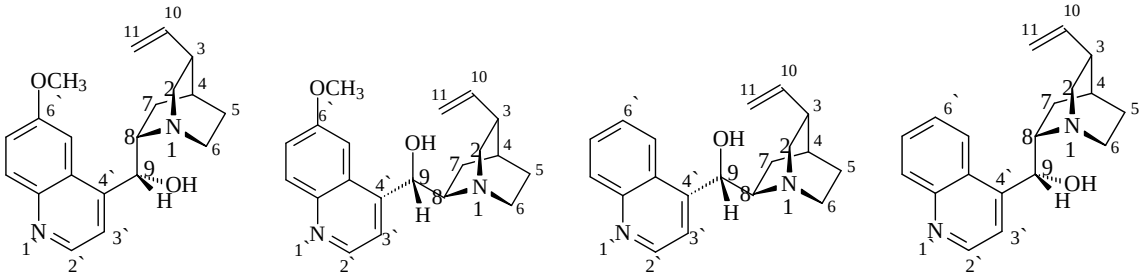
1. INTRODUCERE

Cinchona alkaloids are the most important compound class, being isolated from the *Cinchona* and *Rubiaceous genera tree*. They are organic molecules with an interesting history. They have been used from the early seventeenth century, when they were first introduced in Europe after the discovery of the antimalarial properties of Cinchona tree bark extract and the isolation of active principles by PJ Pelletier and J.B.Caventou in 1820 [13.14]. About 30 compounds are extracted from the bark of trees. Alkaloid content of the crust ranges from 5 to 16%. The main components of the extract are quinine **1** (60-85%), quinidine **2**, cinconine **3** and cinconidine **4**. For over 300 years cinchona alkaloids have played an important role in medicine and more recently in organic synthesis.



Chinina **1** Chinidina **2** Cinconina **3** Cinconidina **4**

Figura.1.1. Structure of cinchona akaloids

Cinchona alkaloids are structurally composed of a quinoline ring and linked to a bulky heterocycle, chinuclidinic, connected through a carbon atom to a C9 OH group (Figure1.1). This is one of the four chiral centers of the molecule. Only C8 and C9 can have different configurations. C8 and C9 chiral centers are S and R in quinine, quinidine, respectively both R and S isomers being eritro. The epimers of these compounds are 8S and 9S for epiquinine respectively 8R and 9R for epiquinidine, these compounds are threo isomers [22].

Hoffmann and co. were able to split the cinchona alkaloids in pure enantiomer quinoine derivates, 1-azabiciclo [2.2.2] octane. This was achieved trough the oxidation reaction while simultaneously reducing exposure to air. The reaction takes place in ether or tetrahydrofuran [33]. During investigations they have tested various reducing agents, the best results were obtained when using lithium aluminumhydride. The fact that this reaction takes place only

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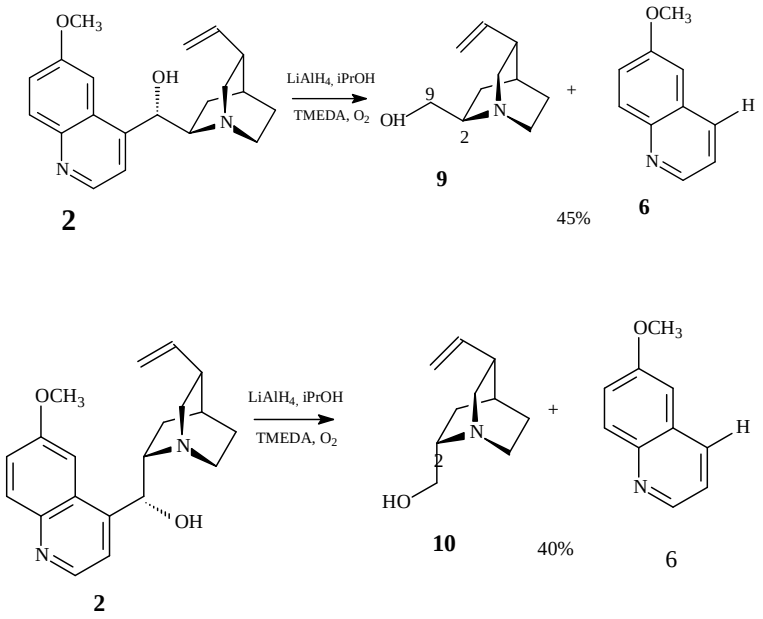
if the reduction occurs in the presence of an oxidizing agent, suggests that the mechanism is more complicated (Scheme 1.1) [34].



Scheme 1.1.

In the last two decades, cinchona alkaloids appeared as chiral auxiliaries being a landmark in asymmetric synthesis. More recently, it has been proven that cinchona alkaloids can be a subject to remarkable skeletal changes, rapidly expanding their chemical perspective.

The key characteristic responsible for cinchona alkaloids successful derivatization is that they have a chiral skeleton with multiple functional groups, multiple transformations being possible.

C9 linked hidroxil group may suffer various reactions, that are characteristic to this functional group: esterification reactions, the substitution of the –OH group and cycle extentions. The practical importance of some products resulting from substitution at C9, has held our attention and has oriented our research to the synthesis of new products [166].

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2. PERSONAL CONTRIBUTIONS

In the personal contributions we followed the points:

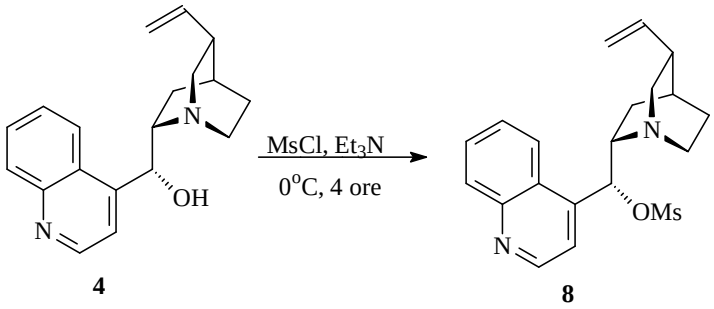
- Synthesis of cinchona alkaloid new derivates trough substitution reactions
- Synthesis of cinchona alkaloid new derivates truncated trough esterification reactions
- Optimization of the reactions used to obtain cinconidine from cinconine
- The development of HPLC cromatographic methods for the synthesized byproducts

2.1. The synthesis of cinchona alkaloid derivates by substitution reactions

In this chapter we present the synthesis of some cinchona alkaloid derivates trough derivtization of the C9OH group..

2.1.1.Synthesis of 9-metansulfonyloxi derivatives of cinchonine and cinchonidine

By studying the substitution reactions at C9 carbon, it was determined that the O-mesil derivate can be obtained under mild circumstances, with good yields. The O-mesil derivate of the cinchona alcaloids activates the molecule, facilitating the substitution reactions at the C9 carbon atom. The synthesis method was the one already described in the literature [100], by the reaction between cinchonidine and methansuphonic acid chloride (MsCl) in the presence of triethyl amine (scheme 2.1)



4 **8**

Scheme. 2.1

To obtain [3S,4S,8S,9R]-Methansulfonyloxi-5-vinyl-cinchonan **8** (O-mesylcinchonidine), 1 mol of cinchonidine **4** reacts with 1,5 mol MsCl using THF as a solvent. The reaction takes place in the presence of triethylamine, the molar ratio cinchonidine:Et_3N 1:2,2 mol (scheme 2.1. The completion of this reaction was monitored by thin layer cromatography, the eluent being MTBE:Metanol 3:1. The reaction product are purified by column cromatography. O-mesil-

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cinchonidine **8** was obtained as beige acicular crystals and was characterized trough H-RMN, IR and elemental analysis.

Experiments have been made for the optimization of the O-mesilcinchonidine 8, by varying the molar ratio of the reagents, cinconidină:MsCl. The results are presented in table 2.1

Tabel 2.1. The reaction conditions for obtaining O-mesilcinconidinei **8**

Nr. Probă	**Raport molar Cd: MsCl**	**Solvent**	**Timp de reacție (ore)**	**Temp (°C)**	**Rand. (%)**
1	1:1	THF	3	0	56
2	1:1,3	THF	4	0	72
3	1:1,5	THF	4	0	80
4	1:1,8	THF	5	0	82

Synthesis yield of 80% was obtained when the reaction occured with an excess of 50% MsCl of 1:1,5, the reaction duration was 4 hours. If the reactants ratio grows to 1:1,8, the yield does not grow significantly, to 82%, an extension of the duration being necessary (5 hours).

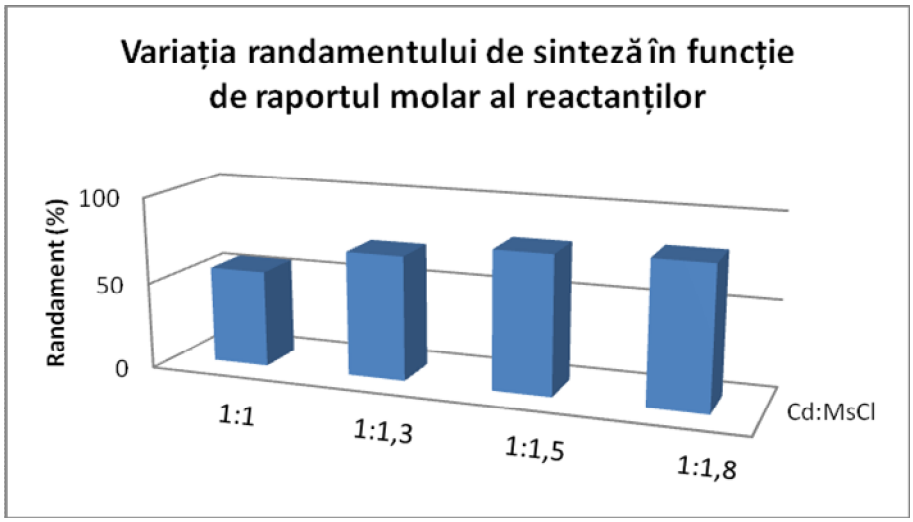

Figura 2.1. The variation of the synthesis yield of compound **97** according to the reactants ratio

In IR spectrum we pointed out the characteristic bands for the SO_2 groups (esters of sulphonic acids) trough the bands present at 1325 and 1139 cm^{-1}.

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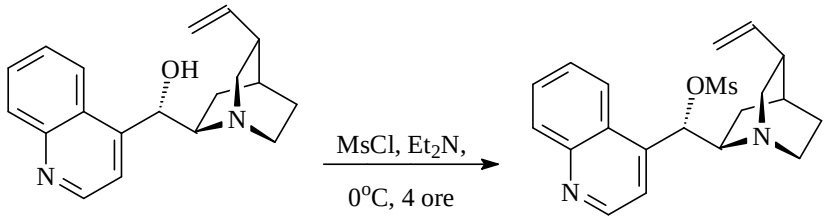
Synthesis of [3R,4S,8S,9R]-methansulfonyloxi-5-vinyl-cinconan (O-mesylcinchonine) **9** was performed by the reaction of cinchonine **3** with MsCl in the presence of triethylamine,[141] the reaction is presented in scheme 2.2.



3 **9**

Scheme 2.2

For obtaining O-mesylcinchonine **9**, 1 mol cinchonine (CN) **3** reacts with 1,5 moli mesylchloride (MsCl) in tetrahydrofuran (THF) used as a solvent. The reaction takes place in the presence of excess triethylamine (TEA) (CN:TEA=1:2,2 moli). The end of the reaction will be verified by thin layer cromatography. The reaction products are purified by column cromatography. After the evaporation of the solvent we obtain a reaction yield of 76,6%. O-mesylcinchonine appears like beige acicular crystals. The product was characterized by H-RMN, IR and elemental analysis.

In order to determine the optimum ratio of reactants some experiments were performed using various quantities of reactants. The results are presented in table 2.2, figure 2.2.

Table 2.2. The reaction conditions for obtaining O-mesilcinconinei **9**

Nr. probă	**Raport molar CN: MsCl**	**Solvent**	**Timp de reacție (ore)**	**Temp (°C)**	**Rand. (%)**
1	1:1	THF	3	0	49
2	1:1,3	THF	4	0	63
3	1:1,5	THF	4	0	76
4	1:1,8	THF	5	0	79

The best results were obtained when experiments were performed with a molar ratio of reactants cinconina:MsCl of 1:1,5. By increasing the quantity of MsCl, above an excess of 50%, we do not obtain a significant increase in yield. In the same reaction conditions we obtainedan

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80% yield for O-mesilcinconidinei **8** and 76 % for O-mesilcinconinei **9** (76%) due to its structure.

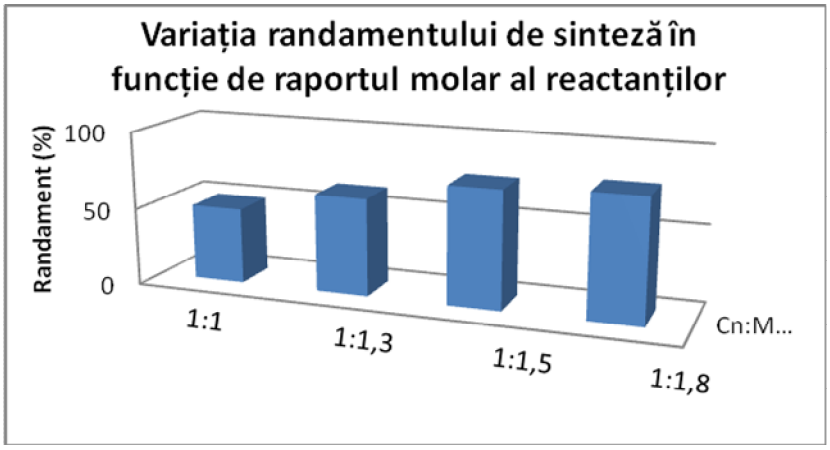


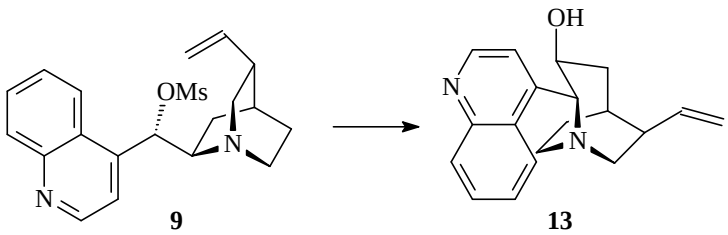
Figura 2.2. The variation of the synthesis yield of O-mesilcinconinei **9** according to the molar reactants ratio

The structure of the synthetized compound was confirmed, it was possible to reveal the specific bands of S = O groups at 1325 cm^{-1} și 1139 cm^{-1} in IR spectrum.

2.1.2. Synthesis of 3-hidroxi-2-(chinolin-4-yl)-6-vinyl-1-azabiciclo[3.2.2]nonan

Cinchonine **3** și cinchonidine **4** with their natural C9 configuration, can suffer, in some conditions, some transformations called order II transpositions. [130,131].

By studying the properties of O-mesilcinchonine **9** it was found that it is slighty soluble in cold water but its solubility increses significantly in hot water. After heating we observed the conduct of the cage expansion reaction and forming of β-substituted amines, (1S,2R,5R,6R)-3-hidroxi-2-(chinolin-4-yl)-6-vinyl-1-azabiciclo[3.2.2]nonan, **13,** keeping the configuration of the chiral carbon atoms. (schema 2.3)



9 **13**

Scheme 2.3

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The reaction was conducted with an diluted solution of O-mesilcinchonine **9** (10% concentration), at reflux, in a nitrogen atmosphere, using pure water. The end of the reaction was verified by thin layer cromatography. The purification of **13** compund is done on a silica gel columnn, using MTBE:MeOH=3:1 as a mobile phase. The obtaining of the product was confirmed by H-RMN, IR spectrometry and elemental and mass analisys.

Beginning from the premise that the reaction is not favorized by the presence of the protons in the reaction environment[147], we obtained methasulphonic acid as a secondary product. We followed the infuence of the basic compound addition over the synthesis yield of compound **13**. For this study we added basic compounds, in order to neutralize the methansulphonic acid that was formed in this reaction. We followed the influence of the following compounds: $NaHCO_3$, Na_2CO_3, NaOH, sodium benzoate. The carbonates generate hidroxyl trough water hydrolysis, the same happens if we dissolve Na OH in water. The sodium benzoate was introduced in the reaction mixture because the literature presents data on the influence of these compounds in the transposition reaction of the cinchona alkaloids. [130] Results are presented in Table 2.3, figure 2.3.

Table nr.2.3. The reaction conditions of compound **13**

Proba	**Bază**	**Concentrație (%)**	**Timp (ore)**	**Randament (%)**
1	$NaHCO_3$	8	16	23
2	Na_2CO_3	10	5	39
3	NaOH	5	3	52
4	BeNa	10	4	30
5	Apă	-	4	51

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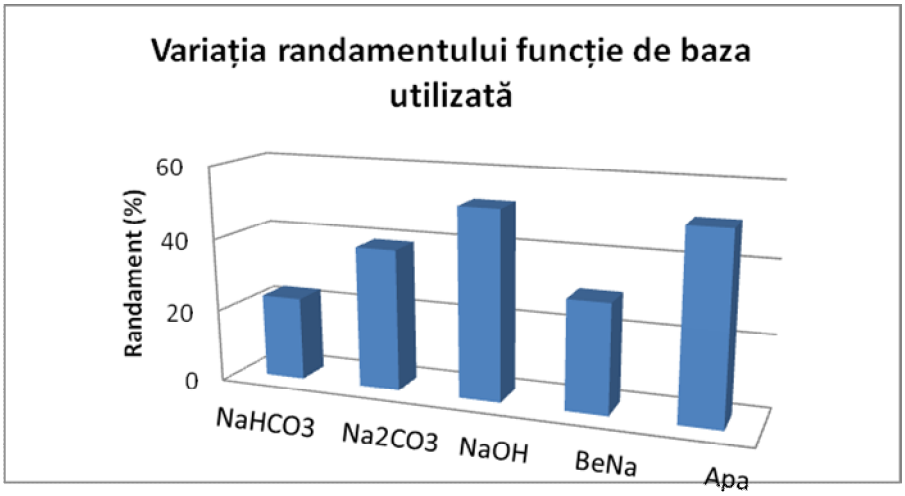
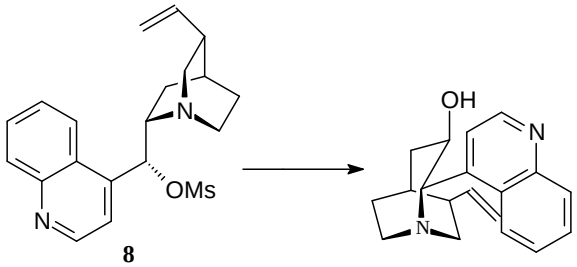


Figura 2.3. Yield variations according to the base used in the synthesis of compound **13**

Good results are obtained if the reaction is carried out in NaOH soution and pure water, the yields were almost equal. If the reaction occurs with only pure water, the basic character of the tertiary nitrogen atom has a positive effect over the reaction. Due to advantages of using only water as a reactive, being practically a process belonging to ”green chemistry”, this was of working is considered optimal. In the IR spectra there were revealed characteristic bands for the vibrant C - O bond, 1227 cm^{-1} and for the in plan stretching O – H bond at 1338 cm^{-1}. We could highlight in the mass spectra the base peak 293 and the isoelectric peak 295. These peaks confirm the molecular mass of the compound.

The literature shows that O-mesylcinchonidine **8** can react similarly, the transposition taking place with the cycle expansion. (Figure 2.4)



8 **14**

Figure 2.4

The reaction was carried out with a diluted, aqueous solution of O-mesilcinconina **8** (10% concentration), at reflux, in a nitrogen atmosphere. The end of the reaction was monitored by thin layer cromatography, the eluent being MTBE:Methanol 1:9, UV detection. The purification of

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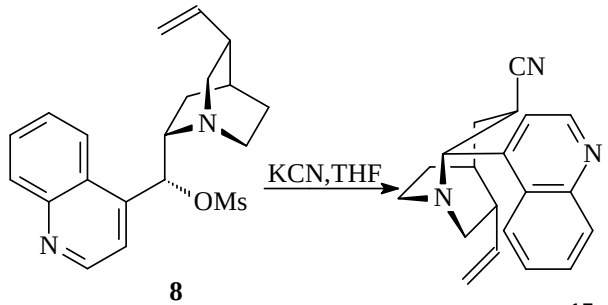
the compound **14** was achieved by column cromatography on silica gel filling. In this case the yieds are higher compared to thoseobtained when the substrate is a derivative of cinconine **14**, and it can be explained by the steric promotion of the reaction (62% compared to 51%). The obtaining of these compounds was examined by H-NMR spectrometry, IR, mass and elemental analysis. The IR spectrum showed the presence of the –OH secondary group, trough the C – O characteristic vibration bands 1252 cm^{-1}, and O – H, stretching bands, 1395 cm^{-1}.

2.1.3. Synthesis of 2-chinolin-4-yl-6-vinyl-1-aza-biciclo[3.2.2]nonan-3-carbonitril

Reactions that form new C – C connections are important for organic synthesis. One possible reaction which can increase the chain with a carbon atom is the synthesis of nitriles and the subsequent derivatization of the synthetizes compounds. For this reason we performed a transposition reaction in the presence of cyanide ion as a nucleofil. (Scheme 2.5).

Synthesis of 2-quinoline-4-yl-6-vinyl-1-aza-bicyclo [3.2.2] nonane-3- carbonitril **15** was achieved by reaction between O- mesylcinchonine 8 and KCN (excess), according to scheme 2.6.

The reaction was carried out in 2,2,2-trifluoroethanol solvent (THF) with a molar ratio of reactants O-mesylcinchonidine **8**:KCN of 1:3 in nitrogen atmosphere at a temperature of 89-90 ° C for 3 days. The end of the reaction is monitored by thin ayer chromatography on silica gel plates using MTBE: MeOH 3:1 as an eluent. Compound **15** separation was achieved by liquid chromatography on a silica gel column using ethyl ether: methanol 9:1 as an eluent.



8 **15**

Schema 2.5

The modest reaction yield, of only 59.5% is due to lower nucleophylicity of the cyanide ion. If this reaction was performed with the same molar ratio of reactants, introducing triethylamine (O-mesilcinconidina: Et3N = 1:1) we obtained the desired product, as an intense

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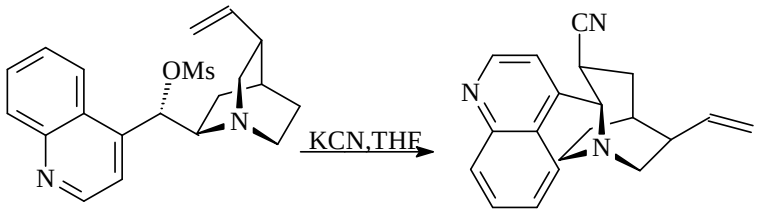
yellow liquid, but with a lower yield, of only 46.2%. the obtaining of the compound was examined by H-NMR spectrometry, IR and elemental analysis

The reaction that used O-mesylcinchonine as a substrate, with nucleofilic potassium cyanide was performed under the same conditions, resulting nitrile **16**. (Scheme 2.6)



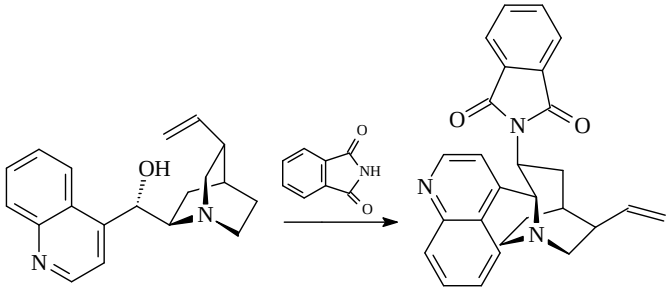
9 **16**

Scheme 2.6

Synthesis yield of compound **16** is 43.14%, the lower yield explained by steric hindrance of the substrate. The product was characterized by NMR spectroscopy and elemental analysis H-RMN and elemental analysis.

2.1.4. Synthesis of 2-(Chinolin-4´-yl)-6-vinyl-1-azabiciclo[3.2.2]nonan-3-yl-amine

In 1972, Mitsunobu presented a derivatization reaction of primary and secondary alcohols in the presence of nucleophylici, for example compound with nucleophylic nitrogen. The reaction takes place successfully using the nucleofil, phthalimide. We performed this reaction using the substrate O-mesiylcinchonine **9** (Scheme 2.7)



9 **17**

Scheme 2.7

The reaction occurs in the presence of t-butylamine, the solvent is 2,2,2-trifluoroethanol. Separation of the compounds in the reaction mass was performed by column chromatography on silica gel filling, the eluent being diethyl ether-methanol mixture 9:1.

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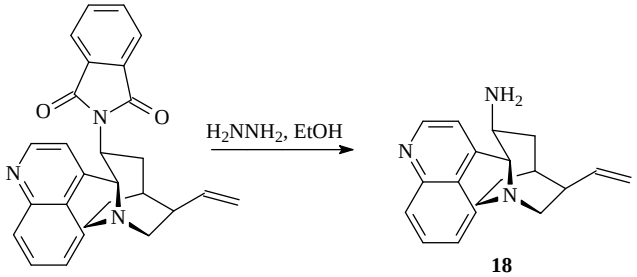
To separate the pure reaction product we had to take two successive separations. Reaction efficiency is low, only 17.5 %. The compound (2-(quinolin-4'-yl)-6–vinyl-1-azabiciclo [3.2.2] nonan-3-yl)-phthalimide **17** synthesized on this manner was characterized by ^{1}H-NMR spectroscopy and elemental analysis.

By reducing compound 17 with hydrazine in an alcoholic medium, we obtain an aminoderivate corresponding **18** white crystals, yield 52%, according to scheme 2.8.



17 **18**

Schema 2.8

The reaction was performed with an excess of hydrazine, molar ratio 17: hydrazine = 1:1,3moles in anhydrous ethyl alcohol. Reaction time was 50 hours, the reaction was monitored by thin layer chromatography, the eluent was ethyl ether-ethanol-ammonia 25% 50:12:1 . Amine 18 was separated from the reaction mass by column chromatography. The finished product, acicular white crystals, was characterized by H-RMN spectroscopy and elemental analysis.

2.1.5. Synthesis of methansulphonic acid esters of 3-hidroxi-2-(chinolin-4-yl)-6-vinyl-1-azabiciclo[3.2.2]nonan

OH group can easily undergo esterification reactions. The literature indicates that in order to achieve substitution reactions, it is necessary that cinchona alkaloids molecule to be first activated by a transformation to an ester, such as with methanesulphonic acid. Methanesulfonic acid chloride reaction undergoes in relatively mild conditions with a good yield. In order to neutralize the hydrochloric acid formed during the reaction (Scheme 2.9), the reaction takes place in the presence of triethylamine. We used an excess of chloride mesylate, molar ratio alcohol: MsCl = 1:1,5. The Mesilderivaţii thus obtained can be subjected to substitution reactions, OMs group being easily replaceable.

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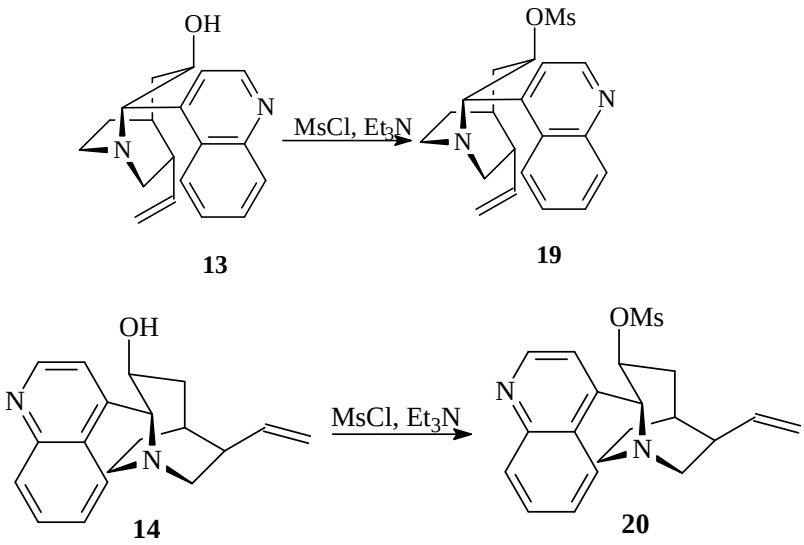
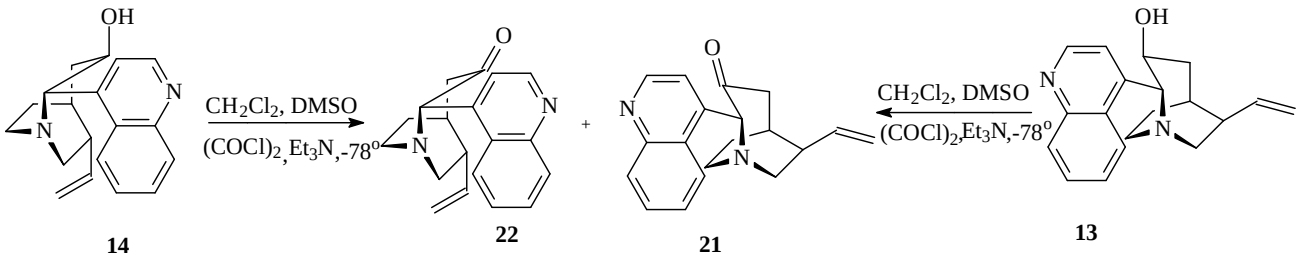


Scheme 2.9

During the reaction, hydrochloric acid is formed as a secondary product and it is necessary to add triethylamine to shift the balance towards the synthesis of the desired product. We obtain O-mesylate derivatives in the form of yellow acicular crystals, yield 65.65% for compound **19** and 36.8% respectively for compound **20**. Obtaining the compound was confirmed by H-RMN spectrometry and elemental analysis.

2.1.6. The oxidation reaction

Reaction with dimetilsulfoxid (DMSO) in the presence of electronic activators (Swern oxidation) proved to be a gentler oxidation method highly used for converting alcohols to carbonylic compounds. Swern oxidation [165] of β - aminoalcoolilor **13** and **14** leads to the formation of a mixture of epimeri, α-aminocetonelor azabiciclici **21** and **22** in 2:1 molar ratio (Scheme 2.10)



Schema 2.10

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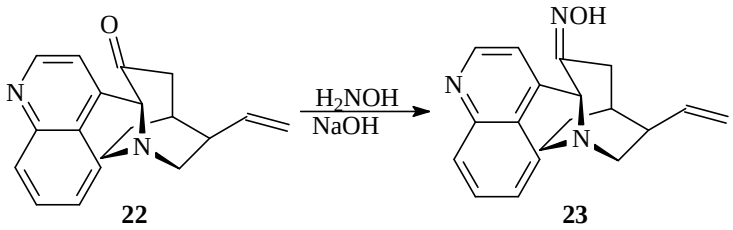
Oxidation reaction was performed with a molar ratio 1:1 chloride oxalil and DMSO, the solvent was CH_2Cl_2. The reaction takes place at a temperature of -78 ° C. Either hydroxylated compound is subjected to the oxidation reaction, **13** or **14** it is measured in the reaction mass at a molar ratio of 1:2 against the oxidation agent. The separation of the reaction mass and the purification of the ketone was achieved trough liquid cromatography, on silica gel column packing, eluent MTBE: MeOH 9:1. Ketone **22**, separated and purified was characterized by H-NMR spectrometry, IR and elemental analysis. The presence ofbands at 1633 cm-1 and 999 cm-1 in the IR spectrum indicates the presence of the corbonil group of the prepared ketone.

It was noticed that the baance between compound **22** and **21** remains constant. If compound **22** which is separated by liquid chromatography is kept in the laboratory at room temperature for 48 hours, it partially transforms to compound **21**, respecting the **ratio 22:21** 2:1.

2.1.7 Condensation reactions with nitrogen compounds

By treating ketones with hydroxylamine salts (hidrochoride), in aqueous solution, eventually with a mild heating, oximes are formed. In the reaction of ketone **22** with hydroxylamine hydrochloride in excess (1:10) we obtain the corresponding oxime **23**, with a yield of 37,95% (scheme 2.11). The reaction occurs in the presence of solid NaOH at refux. The oxime purification was achieved trough column cromatography.



22 **23**

Scheme 2.11

HRMN spectrometry, IR and elemental analysis confirmed the structure of newly synthesized compound. The presence of 1693 cm^{-1} bands, 1494 cm^{-1} shows the presence of C = N bond, characteristic to these compounds.

Condensation reaction can occur with other compounds containing amino group. In the reaction with glycine methyl ester (Scheme 2.12) we obtain compound 24. This new compound can be further derivatized, synthesizing peptides, potentialy biologically active compounds.

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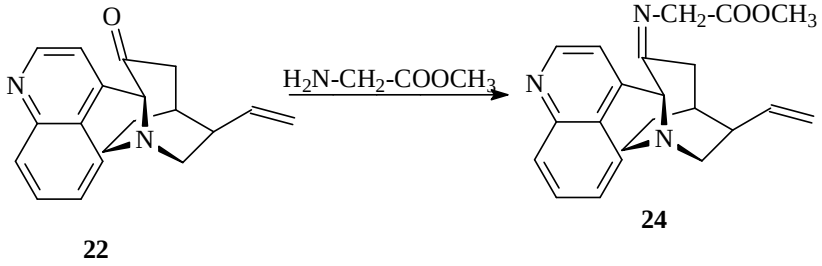


22 **24**

Schema 2.12

Molar ratio of reactants used in this synthesis was ketone: methyl glycine 1:1. The reaction occurs in the presence of p-toluensulphonic acid in toluene. Water, a byproduct of this reaction is removed from the reaction mixture by azeotropic distillation. Reaction course is monitored by thin layer chromatography, eluent $CHCl_3$: CH_3OH 9:1 and by observing the resulting water. We obtained 0.07 g of white crystals, with a yield of 37.8%. Obtaining the compound was examined by H-NMR specrometry, IR, mass and elemental analysis.

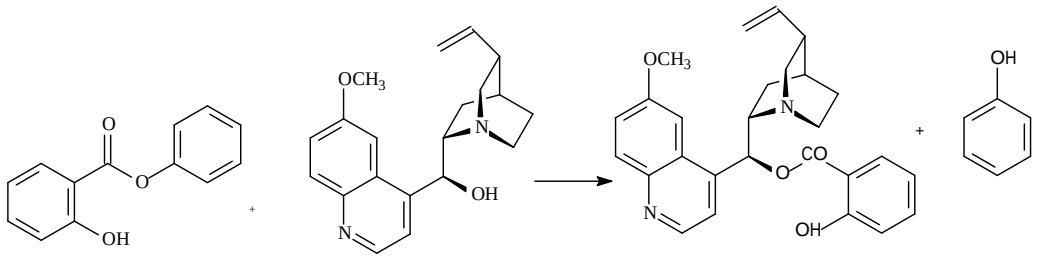
2.2. Synthesis of new esters of QCI și QCD

The discovery of new drugs is an extreme challenge, for scientific and economic reasons. To create a new drug it takes about 12-15 years and it requires a budget of 0.8 to 1.7 billion dollars [167], 10% of which is attributed to synthesis studies, 70% being used for preclinical and clinical studies.

Natural product chemistry has lately been revised and they will continue to be an important source of drugs [168]. Half of the drugs currently in clinical trials are derivates from natural compounds [169].

Esters may be prepared by reactions between a carboxylic acid and an alcohol in the presence of catalysts: sulfuric acid, benzenesulfonic acid, or hydrochloric acid gas. This reaction is called Fiescher esterification (E. Fiescher 1852-1919). The most common obtaining reactions for esters and amides are O-and N-acylation, and they use as acylating agents acyl chlorides or anhydrides. They react rapidly with deactivated alcohols. Because the acyl halides are reactive to weak nucleophylic, such as alcohols, the reaction may result without requiring catalysts.

In the early 1900s quinine ester with salicylic acid was synthesized and it was called salicilchinina by reaction of salol and quinine. (Scheme 2.13)[90]



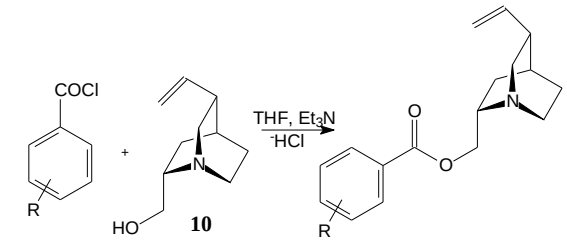
25 **1** **26** **27**

Schema 2.13

Salicylquinine 26 so prepared has antipyretic and anagesic properties. These considerations have directed research for synthesizing new compounds, esters and amides of cinchona derivatives.

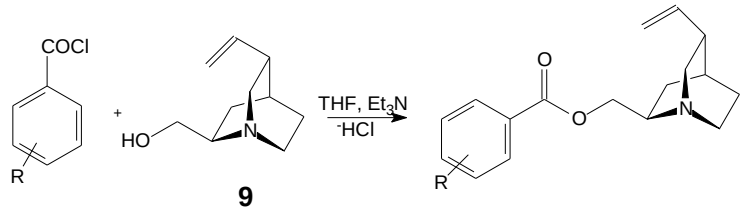
2.2.1. Synthesis of esters of 1,2 aminoalcohols QCI și QCD

New compound, esters of benzoic acid, salocylic acid and acetylsalicylic acis with 1,2-aminoalcohols QCI **10** and QCD **9** were synthesized by esterification reaction using acid chlorides as acylating agents for the alcohols [177,178]. (schema 2.14).



28 R = H
29 R = OH
30 R = $OCOCH_3$

31 R = H
32 R = OH
33 R = $OCOCH_3$



28 R = H
29 R = OH
30 R = $OCOCH_3$

34 R = H
35 R = OH
36 R = $OCOCH_3$

Scheme 2.14

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To improve the yield of the ester synthesis, there were performed experiments in order to optimize the synthesis of ester **31**. For this we studied the influence of the molar ratios of 1,2-aminoalcoolul: benzyl chloride 28 and 1,2-aminoalcool: TEA on the yield. In table 2.4 and figure 2.4 there are presented the results obtained with the variation of 1,2-aminoalcool: benzoyl chloride moar ratio.

Tabel 2.4. Influența raportului molar 1,2-aminoalcool:clorura de benzoil

Nr. Exp.	alcool		Clorura de benzoil (mmoli)	TEA (mmoli)	Randament (%)
	Tip	Nr.moli (mmoli)			
1	QCD	1	1	2	62,3
2	QCD	3	5	6	83,8
3	QCD	1	2	2	85,1
4	QCI	1	1	2	60,1
5	QCI	3	5	6	84,5
6	QCI	1	2	2	83,9

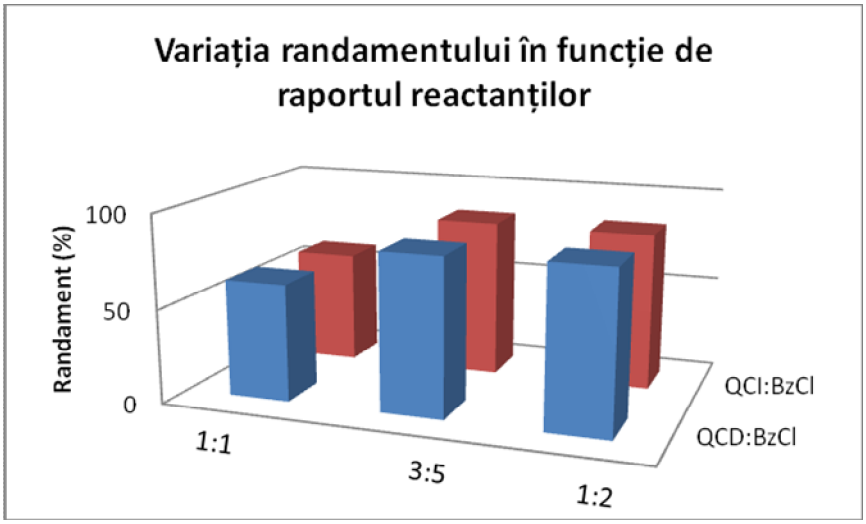

Figura 2.4. Influence of molar ratio 1,2-aminoalcool: benzoyl chloride

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During this experiments it was observed that when using a molar ratio QCD: BzCl = 1:1the yield has a value of only 62.3%, which is the lowest. When the molar ratio QCD:BzCl is 1:1,66 yield increases to 83%. If using a molar ratio QCD:BzCl of 1:2 we do not observe a significant increase in yield (85%). Based on the results (table 2.5, figure 2.5) we considered 1,2-aminoalcool: BzCl of 3:5 an optimal molar ratio. An increase in the molar ratio of 1:2 is not economically optimal. Table 2.5 presents the influence of the 1,2-aminoalcohol:TEA ratio over the synthesis yield.

Tabel 2.5. Influența raportului molar 1,2-aminoalcool:TEA

Nr. Exp.	Alcool		Clorura de benzoil (mmoli)	TEA (mmoli)	Randament (%)
	Tip	Nr.moli (mmoli)			
1	QCD	1	1,66	1	53,6
2	QCD	1	1,66	1,5	74,2
3	QCD	1	1,66	2	83,8
4	QCI	1	1,66	1	50,4
5	QCI	1	1,66	1,5	77,1
6	QCI	1	1,66	2	84,5

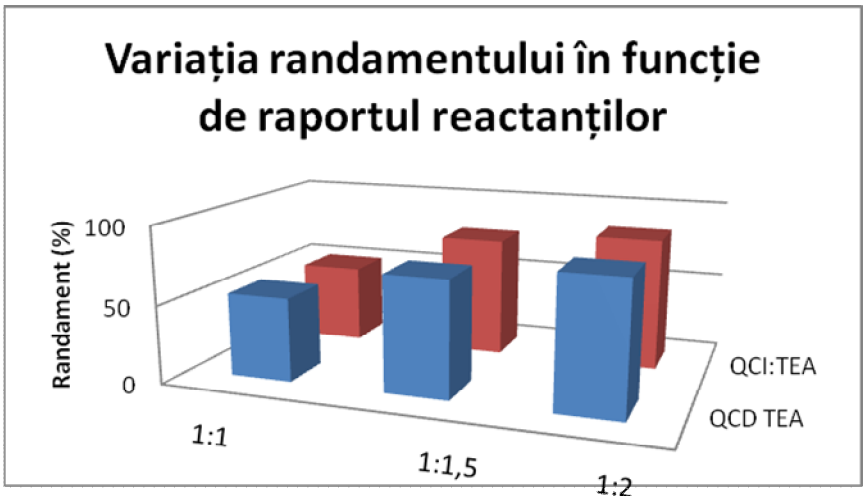

Figura 2.5. Influența raportului molar 1,2-aminoalcool:TEA

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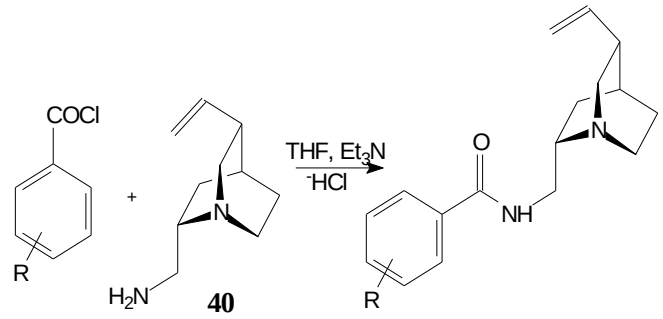
It appears that increasing the molar ratio QCD: TEA from 1:1 to 1:2 we obtain an increase in efficiency of synthesis of the esters by about 30%. The same effect is observed with QCI aminoalcohol. Experiments have shown that for a satisfactory performance it is necessary to carry out the synthesis using reagents in the molar ratio QCD: BzCl: TEA 3:5:6.

There were synthesized the esters of 1,2-aminoalcohols with benzoic acid, salicylic acid and acetylsalicylic acid. It was found that synthesis yields decrease for example from 83,8% for benzoic acid ester to 64,76% for acetylsalicylic acid. Electron attractive substitutes grafted on the benzene core decrease the reactivity of the acyl chloride. The decrease in synthesis efficiency can be explained by the steric obstruction effect.

Newly synthesized compounds were characterized by H-NMR spectrometry, IR and elemental analysis. The presence of 1768 cm-1 bands, 1393 cm-1 are characteristic to benzoate, which proves the desired ester synthesis.

2.2.2. Synthesis of amides of 1,2 diamine QCDNH$_2$

Amides of benzoic acid, salicylic acid and acetylsalicylic acid with diamine QCDNH2 were synthesized using chlorides of the acids as acylating agents for the amines (scheme 2.15).



28 R = H
29 R = OH
30 R= OCOCH$_3$

37 R = H
38 R = OH
39 R = OCOCH$_3$

Schema 2.15

Synthesis of these amides is achieved with satisfactory yields 80.9% for benzoic acid amide **37**, 71.7% for salicylic acid amide **38** and 49% for acetylsalicylic acidamide **39**. There can be noticed the disable effect of electron attractive groups related to benzene nucleus on this reaction, highlighted by the decrease the yield of synthesis.

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It has been studied the influence of the molar ratio of the reactants over the synthesis yield. Table 2.6 and figure 2.6 present the results.

Tabel 2.6. Influence of the molar ratio of the reactants over the synthesis yield

Nr. Exp.	Amina		Clorura de benzoil (mmoli)	TEA (mmoli)	Randament (%)
	Tip	Nr.moli (mmoli)			
1	$QCDNH_2$	3	3	6	44,3
2	$QCDNH_2$	3	4	6	69,7
3	$QCDNH_2$	3	5	6	80,9
4	$QCDNH_2$	3	6	6	82.2
5	$QCDNH_2$	3	5	4,5	71,8
6	$QCDNH_2$	3	5	3	55,6

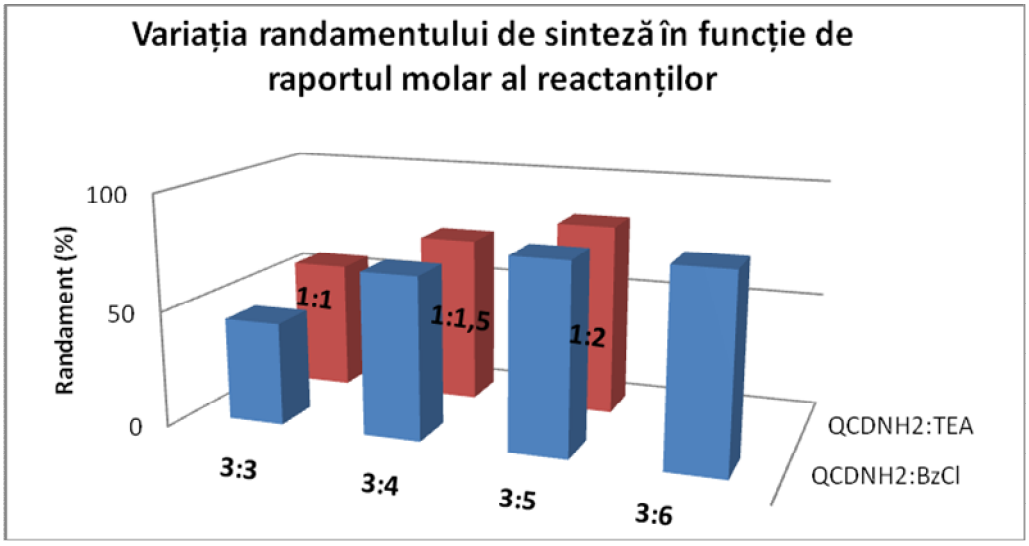

Figura 2.6. Influence of the molar ratio of the reactants over the synthesis yield

During this study we have performed synthesis of the corresponding amides using molar ratios $QCINH_2$: benzoil chloride of 1:1 – 1:2. It was found that if synthesis is carried out using a molar ratio of 1:1 we obtain a yield of 44.3%. By increasing the ratio to 3:5 the yield increase to about 81%. By using a ratio of 1:2, the yield does not increase significantly, we only achieve an efficiency of 82%. For this reason the 3:5 ratio of reactants is considered optimal. Further

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syntheses were performed in which the molar ratio of QCDNH2: TEA varied. The 1:1 molar ratio obtains a yield of 55.6%, and for 1:2 ratio it increases to 80.9% efficiency. Synthesis 3 from table 2.6 presents the optimal synthesis conditions.

Newly synthesized compounds were characterized by H-NMR spectrometry, IR and elemental analysis. In the IR spectrum we observed at 2946 cm the presence of band at 2946cm^{-1}, broadband, 1658 cm-1 characteristic to compounds containing amide groups.

2.3. Study of the optimization of cinchonidine synthesis

From the ancient times, cinchona alkaloids have played a crucial role in the development of organic chemistry and modern medicine. Total synthesis of quinine, in 1945, was regarded as an event of great importance in the development of organic chemistry. Total stereoselective synthesis of quinine, first performed in 2004, represents the pioneering of stereoselectivity in organic synthesis.

The crucial importance of pure enantiomeric compounds for life and science led to a special attention in the research of asymmetric synthesis. The importance of the areas was highlighted in 2001 when the Nobel Prize for chemistry was awarded to chemists W.S. Knowles, R. Noyori, K.B. Sharpless for their results in the asymmetric synthesis field.

Asymmetric reactions using phase transfer catalysts have been studied since the 1970s. The most intensively studied group of catalysts was prepared by cinchona alkaloids quaternization. During this period there have been synthesized the functional derivatives of cinconidinei by derivatization of existing functional groups in the molecule, being intensively used in catalytic asymmetric reactions. Growing demand of cinconidine imposed the study of cinconine transformation processes, a less used compound, in cinconidine.

For this reason, we turned our attention to optimizing the process of synthesis of advanced purity cinconidinei [178].

Cinconidina is a crystalline substance, which crystallises in large, shiny prisms, with a melting point at 204.5° C. It is hardly soluble in water, soluble in alcohol and ether. Sulphuric cinconidine solution does not present fluorescence, does not react to taleoquine, this being a specific reaction for other cinchona alkaloids

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The process of obtaining cinconidine from the stereoisomer cinconine consists of an oxidation of the C9 hydroxyl group in 9-oxo derivative, which is run under Oppenauer method, followed by a stereospecific reduction with borohidrură de sodium.

Stages consist of the following steps:

•Synthesis of cinconidinone;

• Synthesis of cinconidine tartrate;

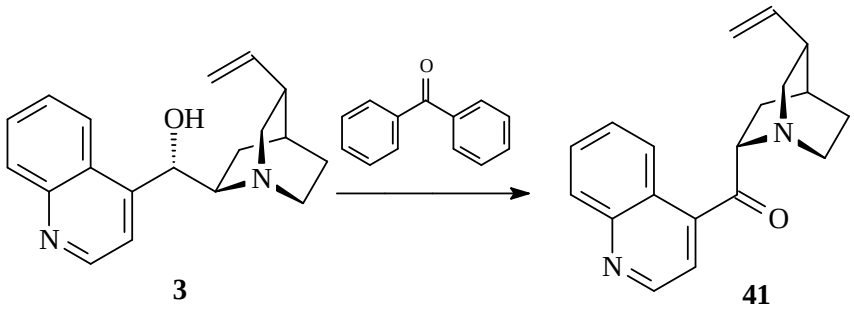
• Synthesis of cinconidine;

• Purification cinconidine

2.3.1. Synthesis of cinchonidinone

There are several methods of oxidation of cinchona alkaloids in the corresponding ketones, particularly for the oxidation of quinine to chininone. Rabe has managed the chromic acid oxidation reaction but yields were low. [179] In 1945, Woodward adapted the Oppenhauer method for quinine oxidation, managing the quantitative oxidation using potassium t-butyrate instead of the classic catalyst aluminum alcohol.[180]

Cinconidinona is obtained by oxidating cinconine applying the modified Oppenhauer method, using benzophenone as an oxidising agent. The occurring reactions appears in scheme Scheme 2.16.



3 **41**

Scheme 2.16

Because cinchonine **3**, raw material, may contain up to 7.5% moisture, and traces of water could lead to undesirable side reactions, it is necessary that cinconina that undergoes the reaction does not contain more than 0.1% water. Drying of cinchonine is conducted by azeotropic distillation with toluene.

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The oxidation reaction occurs in an alkaline medium at a temperature of 90 - 110°C. After completion of the reaction, the formed cinconidona is extracted in aqueous acid solution, from which it is released in the alkalinisation of the aqueous solution to pH = 12 and it is separated by filtration. In table 2.7 there are presented the obtained results.

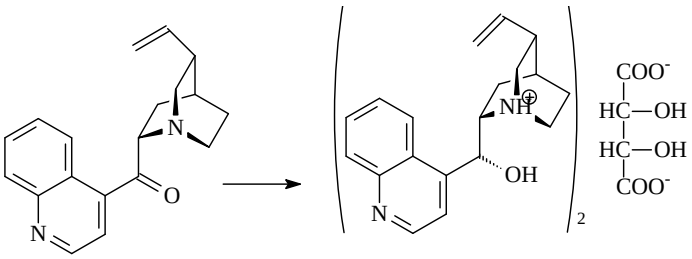
Tabelul 2.7. Optimal conditions of the synthesis process of cinconidinona **41**

Nr. pr	MATERII PRIME					PRODUŞI			
	Cantitate CN + DHCN (g)	Toluen	NaOH + KOH (g)	H_2SO_4 (g)	Benzo-fenonă (g)	CDO (g)	Umiditate (%)	CDO uscat (g)	Randament (%)
1	53,89	415	25+37,1	22	25	81,9	40,1	49,1	91,2
2	55,75	415	25+ 37,1	22	25	86,4	37,2	51,6	92,6
3	55,75	420	25+ 37,1	22	25	86,9	33,3	51,8	91,8

The analysis results show that the oxidation reaction of cinchonine to cinchonidinone using benzophenone as oxidizing agent obtains good yields, from 91.2 to 92.6%. The purity of the obtained product is over 99%.

2.3.2. Reduction of cinconidinona to cinconidine tartrate

Of the many methods of reducing carbonyl group to hydroxyl functional group described in the literature we chose borohidrură sodium reduction due to the work conditions and availability of raw materials. From the reaction mass cinconidine is separated as tartrate **42**. (Scheme 2.17)



Scheme 2.17

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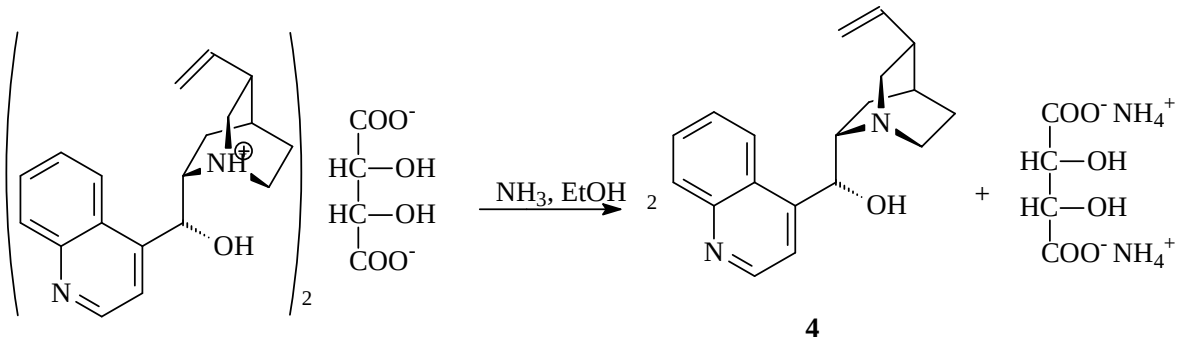
Reduction with sodium borohydride occurs in the environment of isopropyl alcohol at a temperature of - 15 ° C, at pH = 4.5, by alternative dosing of reduction agent and aqueous solutions of tartaric acid. Tartaric acid is used to separate the obtained epimers. The results are presented in table 2.8.

Tabelul 2.8. The results obtained in the synthesis of cinconidinone tartrate 42

Nr pr	MATERII PRIME				PRODUS FINIT					
	CDO uscat (g)	Alc. Izopropilic (g)	Acid tartric (g)	Borohidrură de sodiu (g)	TCD (g)	Umiditate (%)	Conținut bază liberă (g)	TCD (%)	TC (g)	Randament (%)
1	60	120	49	5,3	156	60,12	45,95	94,21	42,72	70,83
2	60	120	49	5,3	112	56,16	41,18	96,5	37,31	61,76
3	60	120	49	5,3	121	56,14	40,74	96,5	39,31	65,08

The reduction reaction occurs with an efficiency of 61-70%, resulting cinchonidine tartrate with a purity of 94-95%, the moisture of the product ranging from 56-60%. Composition was determined by HPLC analysis.

Cinchonidine is released by treatment with alcoholic solution of cinchonidine tartrate, with aqueous ammonia, at a temperature 40 - 50°C. Cinchonidine tartrate is dissolved in alcohol, is heated to 40°C and the ammonia solution is dosed until pH = 10. The product is separated by filtration and purified from the aqueous solution by neutralizing the aqueous solution of cinconidina sulphate. The reaction that occurs is shown in Scheme2.18.



42 **4**

Schema 2.18

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We obtain a product, cinchonidine,appearing as white crystals following the quality requirements of international regulations in force, even as a pharmaceutical product. The obtained results in the synthesis phase of cinchonidine, respectively its purification are presented in Table. 2.9 and 2.10.

Tabelul 2.9. Results obtained in the synthesis of cinconidine **4**

Nr.	MATERII PRIME			Produşi		Conţinut Baze libere (%)
	Alcool etilic (ml)	Tartrat de cinconidina (g)	Soluţie amoniac 25% (ml)	Cinconidina (g)	Filtrat (g)	
1	900	256	60	111	1116	78,1
2	900	256	60	91	1060	83,6
3	900	300	90	109	1046	72,11

Tabelul 2.10. Results obtained in the purification phase of cinconidine **4**

Nr	Materii prime					Produşi	Conţinut (%)	Randament (%)
	Cinconidina		Acid sulfuric 30% (g)	Hidroxid de sodiu 10% (g)	Apa (ml)	Cinconidina Purificata (g)		
	Cant (g)	Conţ (%)						
1	111	78,1	136	433	2500	63	99,86	72,56
2	91	83,6	135	378	2259	55	99,94	72,23
3	120	72,11	193	600	2000	81	99.96	93,6

Following these processes we obtained cinchonidine with a purity from 99.86 to 99.96% with a yield ranging from 72-93%. If working in more concentrated solutions, respectively a smaller amount of water in the reaction medium, yields are higher (93.6%).

For quantitative assay of cinchona alkaloids there are recommended HPLC methods with UVdetectors.

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The necessary eluent for the chromatographic determination is formed from a solution of monosodium phosphate, Triethanolamine, acetonitrile and the pH is adjusted to 2.3 with phosphoric acid.

Qualitative and quantitative determinations were performed using a standard solution containing the key cinchona alkaloids. This standard solution is used to determine retention times characteristic to each compound.

For quantitative and qualitative determinations of cinchona alkaloids a HPLC Able & Jasco chromatograph was used, composed of PU-1580 pump module, ternary gradient module LG-980-02S, DG-980-50 degasser module, manual injector RHEODYNE, detector module UV-1575, data processing programme BORWIN 1.50

For chromatographic separation we used a Nucleosil C18, 250x4.6 mm, 5µ particle column. Detection was performed at λ = 316 nm, wave length of. Figure 2.7 shows a calibration chromatogram.

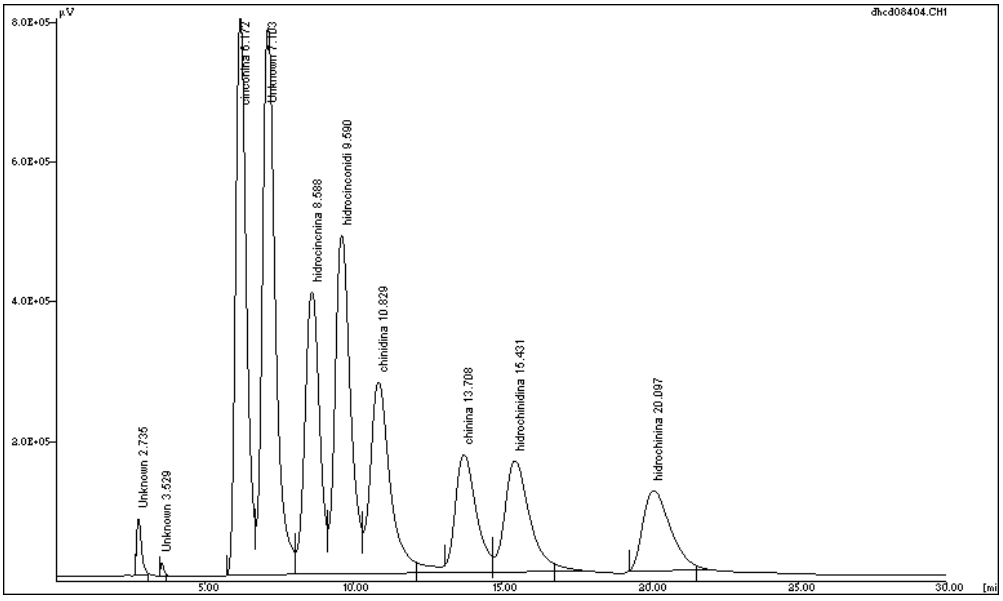

Figura 2.7. Calibration chromatogram, column Nucleosil C18(250x4,6mm), eluent CH_3CH:tampon fosfat, debit 0,7 ml/min, λ=316 nm

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Further considering the sample solution. Identification of compounds derived from cinchona alkaloids shall be based on characteristic retention times, which were determined with the calibration solution.

Sample composition analysis is performed by peak area normalization. Figure 2.8 presents the chromatogram obtained for sample No. 1 from the synthesis of cinchonidinei.

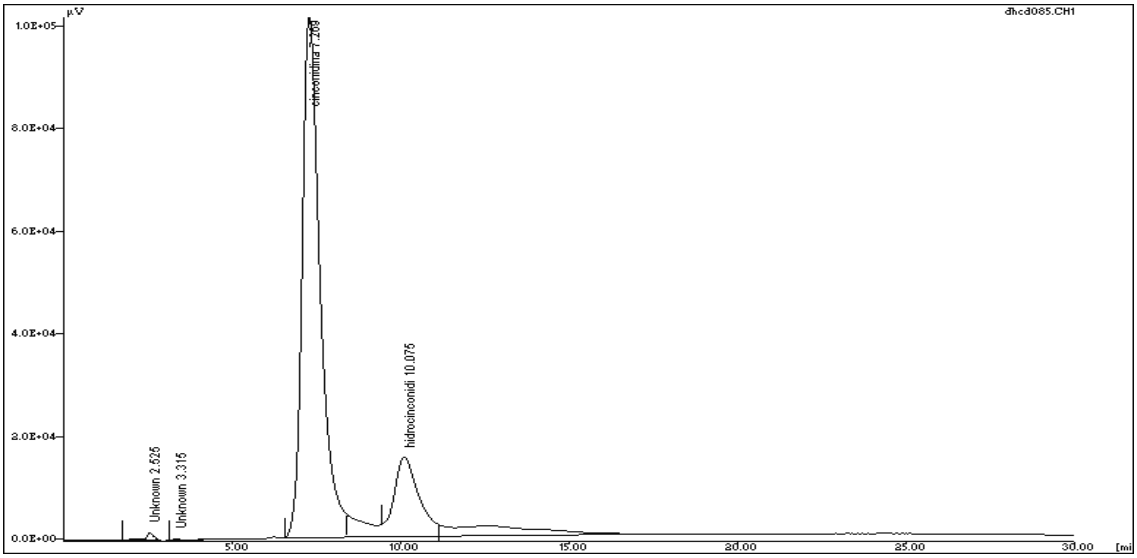

Figura 2.8. Chromatogram for sample No. 1 from the synthesis of cinchonidine, column Nucleosil C18(250x4,6mm), eluent CH_3CH:tampon fosfat, debit 0,7 ml/min, λ=316 nm

Retention times characteristic for cinconidine and dihidrocinconidine are presented in Table 2.11.

Tabel 2.11. Retention times obtained for the standard solution analysis

Compus	RT – soluţia de calibrare (min)	RT-proba (min)
Cinconidina	7,243	7,269
Dihidrocinconidina	9,950	10,07

The results are comparable, the retention times are characteristic to the studied compounds. The determined differences fall within the accepted variations.

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The analysis of the obtained results shows that the samples contain cinconidine and dihidrocinconidine at a rate of 99.84 to 99.96%.

2.4. Elaboration and validation of cromatographic analysis method of QCI-ASA

Isolation and identification of chemicals in mixtures and identification of components has always been one of the priority objectives of chemistry. At present not only in analytical chemistry but also in chemical technology there is an increasing demand for advanced purity compounds. Separation of components of mixtures is an essential operation that allows us to obtain the highest purity. This separation can be done at an analytical scale, if we are interested to know only preparative scale composition of the mixture or if want to get physically separated components.

High performance liquid chromatography covers today approximately 80% of the analysis of molecular organic, organo-metallic and inorganic substances. Together with gas chromatography is an important foothold in modern chemical analysis.

In developing and implementing a method of liquid chromatography, a judicious choice of columns has to be made – of the stationary phase, mobile phase and determination of the influence of temperature on separation.

Validation of a liquid-chromatographic method is designed to verify to what extent the developed chromatographic method corresponds to the purpose. The validation process must consider the following criteria: accuracy, precision, linearity, specificity, limit of detection and limit of quantification.

2.4.1. Development of the analysis method by HPLC cromatography of QCI-ASA

QCI - ASA is a new synthesized compound with a potentially biological action. For this reason we have developed a HPLC chromatographic method for determining this compound [181].

The determinations have been performed using an Able & Jasco chromatograph, equipped with a PU-1580 pump module, ternary gradient module LG-980-02S, DG-980-50 degasser module, manual injector RHEODYNE,detector module UV-1575, Detection wavelength: 254 nm

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Used reagents: hexilamina, potassium hydrogen phosphate, HPLC grade methanol and acetonitrile HPLC grade. They were purchased from Merck company. Doubly distilled water was prepared in the laboratory.Sample preparation: 25 mg QCI acetylsalicylic acid (ASA QCI), weighed accurately, is placed in a 25 ml volumetric flask. Add 10 ml methanol and dissolve the sample. Complete the contents of the flask to the mark with methanol. This is the stock solution. Stock solution has aconcentration of 1mg/ml. For preparation of calibration solutions, dilute the appropriate volume of this solution so that the final solution concentration will be: 50, 40, 25 and 10 μg/ml. All solutions are filtered with a filter unit having a pore size of 0,45 μg.

2.4.1.1. Establishing optimum column for separation

To separate these compounds there have been tested the following columns:

• Nucleosil C 18, 5μm, 250 x 4 mm

• Nucleosil C8, 5μm, 250 x 4 mm

• Hipersil C18, 5μ, 250 x 4 mm

• Lichrosphere RP18, 5μm, 250 x 4 mm

We performed measurements by injecting the sample solution QCI ASA several times, using a mobile phase consisting of: acetonitrile-phosphate buffer (pH 3) 50:50 v / v, solution containing 0.03 mol / l hexilamină, flow 0.5 ml / min.

Each type of column was cromatographed 3 times consecutively, with the solution of concentration 10 mg / ml. For each determination we followed the separation efficiency - the ability of separation and peak shape - asymmetry factor. The results are presented in Table 2.12.

A good separation requires reasonable detention values. Retention time is a direct measure of apprehension. This factor is affected by the fow speed of the mobile phase through the column and the possible interactions with the stationary phase of the sample. Another parameter widely used is "capacity factor" (k), which is equal to the ratio between the retention time of the sample into the column and the column dead time.

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Tabel 2.12. The results obtained at the analysis of QCI-ASA, establishing the optimum column

Nr. Exp.	Tip coloană	Timp retenție (min)	Aria picului (mV·s)	Capacitate k	Asimetrie
1	Nucleosil C18	9,58	87743	915	1,1
2	Nucleosil C8	14,61	75914	1021	1,5
3	Hypersil C18	10,22	89161	942	1,15
4	Lichospher RP18	10,41	86791	969	1,44

If the measurements were made using the Nucleosil C18 column, the results are satisfactory, the retention time is 9.58 minutes, the separation is appropriate, the capacity factor is 915 and asymmetry factor is 1.1 very close to the ideal case, asymmetry factor 1.

The measurements made with Nucleosil C8 column were not satisfactory, retention time is higher and the asymmetry factor (1.5) is much different from the ideal value. Neither in the case of the separations using LiChrospher RP18 column, which contains spherical particles, the results are not satisfactory: 10.41 minutes retention time and asymmetry factor of 1.44.

Measurements have shown that the separation of the new QCD-ASA compound is realised with comparable results both with Hypersil C18 column and NucleosilC18.

2.4.1.2. Establishing the composition of the mobile phase

In the reverse-phase HPLC chromatography, the separation is executed on a non-polar stationary phase and an aqueous mobile phase, moderately polar. With these stationary phases, retention time is great for less polar molecules, while polar molecules elute faster. Retention can be increased by adding water in the mobile phase and can be decreased by adding a larger quantity of organic phase in the eluent. Reverse-phase chromatography operates on the principle of hydrophobic forces, from high symmetry of the water dipole. Analyte binding to the stationary phase is proportional to the contact surface of the non-polar segment of the analyte molecule with ligand particles, in aqueous eluent. This solvofobic effect is dominated by the water capacity of "reduction the cavity" around the analyte and the C18 chain stationary phase. The released energy in this process is proportional to the surface tension of the eluent and hydrophobic surface of the analyte.

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Another important factor affecting the separation is the pH of eluent, because it affects hidrofobicity of the analyte. Usually a buffer component is used for pH control. Buffer substance use has several purposes:

•Controls.the.eluent.pH

• Neutralizes the non-modified silicate groups of the stationary phase

• Act as pair agent for neutralization of the analyte charge

To optimize the separation of new QCI-ASA compound on the Nucleosil C18 column, determinations were performed using mobile phases with different compositions and different pH values.

The nature of the organic phase influences the peak shape of the base substances. We studied the separation efficiency when the mobile phase we used was containing methanol and acetonitrile on Nucleosil C18 column. The results are presented in Table 2.13.

Tabel 2.13. Results obtained in QCI-ASA analysis, establishing the proper mobile phase

Nr. Exp.	Faza mobilă	Timp retenție (min)	Aria picului (mV·s)	Capacitate k	Asimetrie
1	Tampon fosfat (pH 3):metanol 50:50	17,51	58111	1038	1,83
2	Tampon fosfat(pH 3): acetonitril 50:50	9,58	87743	915	1,1

It is noted that when using a mobile phase containing methanol, the separation is not appropriate, retention times are higher, on average 17.51 minutes and peak asymmetry is pronounced, asymmetry factor is1.83. The results obtained when the separations were performed using mobile phase phosphate buffer (pH 3) and acetonitrile are satisfactory, retention time 9.58, 1.1 asymmetry factor.

Peak shape and separation is influenced by the eluent pH. Mobile phase pH can vary between 2-8. During this time the stationary phase in the column is stable . To study the influence of pH on the separation of the substrate, we used mobile phases with different pH values. Determinations were performed with mobile phases with pH values of 3, 5 and 7. The results are presented in Table 5.14.

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A pH = 3, is supported to ensure good stability of reverse-phase chromatography columns. Basic compounds are generally recommended to use a mobile phase with a pH approximately equal to the pKa of the substance.

Experiments have shown (Table 2.13) that the use of a mobile phase containing phosphate buffer, pH = 3 is best suited for the separation of QCI-ASA, the retention times are optimal and asymmetry factor is closest to the ideal value 1.

Table 2.14. Results obtained from analysis of QCI-ASA, establishing optimum mobile phase pH

Nr. Exp.	Faza mobilă	Timp retenție (min)	Aria picului (mV·s)	Capacitate k	Asimetrie
1	Tampon fosfat(pH 3): acetonitril 50:50	9,58	87743	915	1,1
4	Tampon fosfat(pH 5): acetonitril 50:50	20,41	68227	1116	2,05
7	Tampon fosfat(pH 7): acetonitril 50:50	16,13	70046	994	1,56

The buffer solution which is contained by the mobile phase was chosen based on phosphates. Since the peak shape of basic substances is affected by ion exchange interactions, we prepared the buffer solution using potassium monoacid phosphate and not sodium. Buffer concentration is 0.06 M. If the concentration exceeds 0.1 M it is likely that the inorganic salts in the mobile phaseto precipitate. The literature shows that when the eluent contains amine concentration 0.05 M the separation of the basic compounds is improved. To achieve a good separation, longer chain aliphatic amines are recommended because of the hidrofobicity they serve as a column area masking. For this reason, for better separation, we prepared an eluent containing 0.03 mol / l hexilamină.

Another important factor in chromatographic separations is the eluent flow . To establish the optimal value of this parameter experiments were performed using different flow rates of the mobile phase (Table 2.15). Repeated measurements were performed with the working solution, using eluent composition determined in previous experiments. Mobile phase flow rates used in these determinations are: 0.5 ml / min, 0.7 ml / min and 1.0 ml / minute. Tabel 2.15. Results obtained from analysis of QCI-ASA, establishing optimal mobile phase flow

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Nr. Exp.	Faza mobilă		Timp retenție (min)	Aria piculuui (mV·s)	Capacitate (k)
	Compoziția	Debit (ml/min)			
1	Tampon fosfat(pH 3): acetonitril 50:50	0,5	9,58	87743	915
2	Tampon fosfat(pH 3): acetonitril 50:50	0,7	5,25	79543	528
3	Tampon fosfat(pH 3): acetonitril 50:50	1,0	3,62	64211	326

Mobile phase flow for an optimum separation is 0.5 ml / minute. Increasing the mobile phase flow leads to lower retention time and thus determination time, but separation is not appropriate.

Experiments resulted in the establishment of the optimal mobile phase composition which allows efficient separation. It consists of: phosphate buffer: acetonitrile, 50:50 v/v, containing 0.030mol/l hexilamină, pH = 3. pH adjustment was made with phosphoric acid. Figure 2.9 shows a chromatogram obtained under optimum conditions, determined experimentally.

Figura 2.9. Cromatogram QCI-ASA, column Nucleosil C18(250x4,6mm), eluent CH_3CH:tampon fosfat 50:50, pH=3, debit 0,5 ml-min, λ=316 nm

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2.4.2. Validation of the analysis method of QCI-ASA

To assess the quality of an analytical method, determined by its analytical performance, it is needed a validation by analysis of reference substances with this method, followed by comparing the results obtained on the basis of percentage retrievals and calculated relative standard deviation (RSD%) [182].

Validation methodology is designed to demonstrate that an analytical method meets the purpose for which it was developed and that the performance of such a method established by experimental laboratory studies, meet the requirements for its application in determining the analyte considered [183,184].

The validation process needs to specify the method validation parameters that must be followed during this process.

Table 2.16. Performance parameters studied during the full validation of the analysis method

parameters	parameter description
• linearity	• proportionality of the calibration model
• Exactitatea	• Demonstration of the absence of systematic errors accuracy or consistency of results with the true value or the arithmetic mean of results • Ability of the determination of the analyte, with resuts colse to 99-100%
• Precision - Repetability - Reproductibility In the laboratory	• Demonstration of the absence of random within-laboratory errors, or their low value, the results demonstrated a correlation between them - Featuring obtaining accurate results, repeated by the same analyst, shortly - Featuring obtain accurate results, repeated by the same analyst on different days
• Robustness	• Ability to remain unaffected method to small variations in method parameters

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- Limits -The detection -The quantification	• minimum concentration below which the analyte can not be determine • the lowest concentration of analyte that can be accurately dose

2.4.2.1. Precision evaluation

Accuracy is affected by systematic and random errors.

Accuracy is assessed by determining the repeatability and reproducibility of the method applied in all its phases. For this, the established method is applied by a number of times (9 determinations) when it has to obtain sufficient and statistically valid results. To assess accuracy, we calculate the relative standard deviation (RSD) as follows:

$$RSD = \frac{deviația\ standard}{media\ aritmetică} \cdot 100$$

$$deviația\ standard = \sqrt{\sum \frac{|x_i - \bar{x}|}{n-1}}$$

Precision is evaluated by repeated measurements, assessment of the parameters: retention time and peak area. The results are presented in Table 5.17, 5.18.

Tabel 2.17. Results obtained from analysis of solution concentration 0.01 mg / ml

Nr. Proba	**Timp retenție(min)**	**Aria piculuui (mV·s)**	**Număr placi teoretice**	**Asimetrie**
1	9,58	87377	18157	1,12
2	9,56	89432	18034	1,11
3	9,60	86368	18034	1,13
4	9,55	88143	18122	1,08
5	9,54	86421	18111	1,11
6	9,56	84437	18263	1,09
7	9,59	88573	18106	1,09
8	9,57	86548	18077	1,11
9	9,59	85321	18157	1,12
Medie	**9,57**	**86958**	**18118**	**1,11**
DS	**0,0203**	**1586**	**70,77**	**0,016**
RSD(%)	**0,21**	**1,82**	**0,39**	**1,50**

.

Table 2.18. Results obtained from analysis of solution concentration 0,05 mg/ml

Nr. Proba	**Timp retenție(min)**	**Aria picului (mV·s)**	**Număr plăci teoretice**	**Asimetrie**
1	9,57	4362419	18257	1,11
2	9,57	4399107	18024	1,12
3	9,60	4344073	18014	1,13
4	9,58	4357872	18162	1,08
5	9,53	4346227	18101	1,10
6	9,58	4379718	18283	1,09
7	9,59	4331119	18136	1,07
8	9,55	4357788	18107	1,10
9	9,60	4360298	18167	1,13
Medie	**9,57**	**4359847**	**18139**	**1,10**
SD	**0,023**	**20034**	**91,72**	**0,021**
RSD(%)	**0,24**	**0,46**	**0,51**	**1,92**

If the nine experiments, performed consecutively, on the same day, with the QCI-ASA solution 0.01 mg / ml and 0.05 mg / ml obtain retention time of 9.57 minutes with RSD of 0.21% and 0.24% .

Peak area:

•if the solution concentration 0.01 mg / ml is 86 958 mV • s, RSD 1.82%,

•if the solution concentration of 0.05 mg / ml peak area is 4359847 mV • s, RSD 0.46%.

These values indicate the precision of measurements, obtaining statistically valid values, RSD<2%. Experimental values obtained and statistically validated indicate good reproducibility of the method of analysis proposed in terms of small variability of operator parameters.

Reproducibility was evaluated by performing measurements on three consecutive days, for the QCI-ASA solution of 0.050 mg / ml concentration, assessing the accuracy of the statistical interpretation of the obtained experimental values. The results are presented in Table 2.19.

Tabel 2.19. the results obtained at the anaysis of the QCI-ASA solution in 3 consecutive days

timp / arie	**Ziua 1**	**Ziua 2**	**Ziua 3**
1	4362419	4378316	4359721

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2	4399107	4400118	4384745
3	4344073	4393647	4339736
4	4357872	4341122	4366426
5	4346227	4336721	4336429
Medie	**4361940**	**4369985**	**4359911**
RSD	**0,51**	**0,67**	**0,47**

By analyzing the QCI-ASA solution of 0.05 mg / ml concentration in three consecutive days, approximately equal results are obtained with relative standard deviation of 0.47% - 0.67%. Peak area values corresponding to QCI-ASA in the three days of study were statistically analyzed , determining the areas with a relative standard deviation (RSD) of 0.12%. This value is a proof of the robustness of the method,calculated as relative standard deviation well below 2, the maximum acceptable value.

2.4.2.2. Assessment of linearity

To assess linearity the calibration curve is drawn, the change in peak area with the concentration of the solute. It establishes the linearity domain of the method, more exactly the range of concentrations n which the value of analytical signal is proportional to the concentration, so in this area of concentrations the analyte can be determined with reasonable accuracy and acceptable precision.

To assess the linearity prepare solutions of different concentrations: 0.01 mg / ml 0.025 mg / ml 0.04 mg / ml and 0.05 mg / ml QCI-ASA, by successive dilutions of stock solution which has a concentration of 0.1 mg / ml. The solutions are analyzed by repeated injections, the results are evaluated statistically by calculating the relative standard deviation of the obtained analytical response. By plotting the average peak areas obtained depending on concentration, we can determine the calibration curve. The linearity of the segment obtained is evaluated. The results are presented in Table 2.20, Figure 2.10.

Tabel 2.20. Analysis of the QCI-ASA solutions to asses linearity

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Nr. Probă	**Aria piculuі (mV·s)**			
	0,01mg/ml	**0,025 mg/ml**	**0,04 mg/ml**	**0,05 mg/ml**
1	87377	217121	343555	436241
2	89432	219443	341074	439910
3	90368	220173	345431	435407
4	90142	216531	347147	435787
5	86424	218933	342003	434322
Medie	**88749**	**218441**	**343843**	**436335**
SD	**1755**	**15522**	**24789**	**21216**
RSD(%)	**1,98**	**0,71**	**0,73**	**0,49**

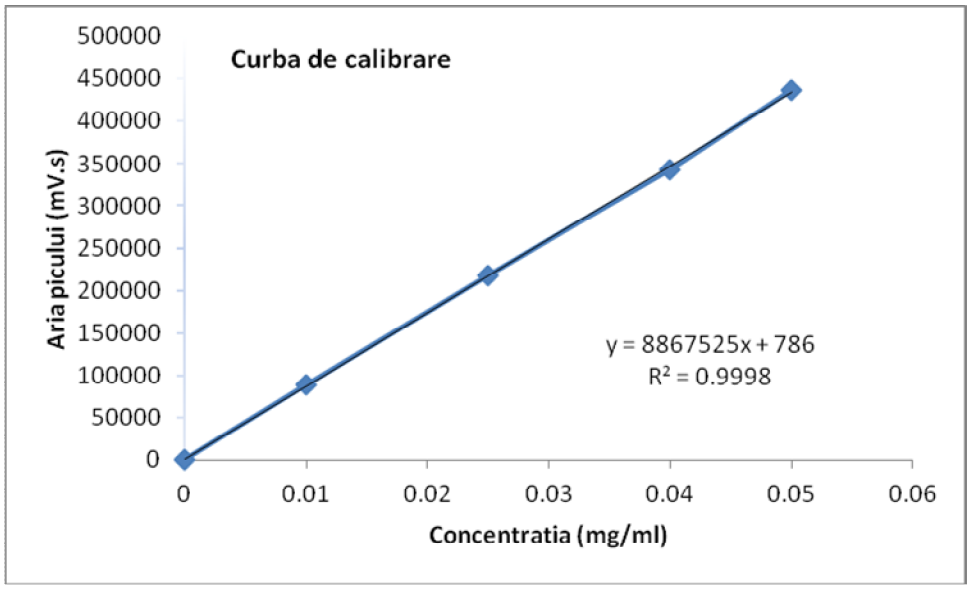

Figura 2.10. Etaonation curve for QCI-ASA

A method of analysis is linear and has no deviation when the slope of the linearity curve passes trough the origin and has a correlation coefficient $R^2 = 0,9998$.

Graphically representing the experimental values we obtain a straight line whose equation is:

$$c\ (mg/ml) = 886725 \cdot a + 786$$

unde: c - concentration

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a – area determined by chromatography of the analyte solution

Table 2.21 presents the results of testing the inearity for the HPLC method .

Tabel 2.21. Results of the linearity tesing

Parametru	**Valori**
Concentration (μg/ml)	10 -50
Regression coefficient(R^2)	0,9998
Slope	886725

In the range of concentration 0.01 mg / ml - 0.05 mg / ml there is a linearity between peak area of QCI-ASA sample and the sample concentration , with a correlation coefficient $R^2 = 0{,}9998 \geq 0{,}99$. A minimum correlation coefficient of 0.99 is the minimum upheld.

2.4.2.3. . Determining the limit of detection and limit of quantification

The limit of detection is equal to the concentration for which the signal analyte /noise is equal to at least 3:1.

Detection limit can be calculated based on standard deviation of the response and slope of linearity_line [ICH]

LD = 3.3 α / P LQ =10 α / P

Where: α - the standard deviation of blank sample

P – slope

To determine the standard deviation of the blank sample, a sample is injected three times . It is prepared similarly to sample preparation and analysis without introducing the analyte. Inject 3 three times this solution and record the noise peak area retention time corresponding to the analyte peak. The results are presented in Table 2.22.

Tabel 2.22. Blank analysis results

Nr. Probă	**Timp de retenție (min)**	**Arie pic (mV·s)**

Thus it was determined:

α = 12.58 mV • s

P = 8867525

Applying the calculation of detection limit and quantification limit we obtain:

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1	9,56	803
2	9,55	778
3	9.69	793
Medie	**9,60**	**791,33**
SD	**0,078**	**12,58**

2.4.2.4. Checking the robustness of the method

Robustness study allows to define allowable variations for the parameters of the critical operators that do not affect the validity of the results provided by the method of analysis.

Given the analysis conditions for the QCI-ASA product we followed the influence of:

- Changes in the mobile phase flow
- Changes in mobile phase pH
- Changes of detection wavelength

The results obtained when varying these parameters by ± 10%, are presented in Table2.24

Tabel 2.24. Method robustness assessment results

parametru / conc	**Valoarea concentrației de analit regăsit**					
	Debit		**pH**		**Lungine de undă**	
	0,5 ml/min -10%	**0,5 ml/min - 10%**	**3 – 10%**	**3 + 10%**	**254nm –10%**	**254 + 10%**
1	0,47	0,50	0,52	0,50	0,50	0,51
2	0,47	0,51	0,51	0,52	0,51	0,50
3	0,49	0,51	0,51	0,51	0,50	0,50
Medie	**0,476**	**0,506**	**0,513**	**0,51**	**0,506**	**0,503**
ER(%)	**4,8**	**1,2**	**2,6**	**2,9**	**1,2**	**0,6**

In each case the relative error is calculated for these measurements using the relation:

$ER(\%) = (A-0,5)/0,5 \cdot 100$

Errors vary between 0.6 - 4.8%. The results with the highest error are obtained when varying the mobile phase flow. If the mobile phase pH changes, relative errors of determination

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of QCI-ASA content ranges from 2.6 to 2.9. Wave length variation has the smallest influence in this determination.

Experimental values obtained show that the proposed analytical method is accurate, allowing quick and accurate determination.

3. CONCLUSIONS

- 8 new products were synthesized, obtained by SN2 substitution reactions of cinchona alkaloids - cionconina and cinconidina. Newly synthesized compounds are derivatives of 1-azabiciclo [3.2.2] nona. New sinthesized products were characterized by H-NMR spectroscopy, IR, MS and elemental analysis. These compounds are precursors for new catalysts for asymmetric reactions and phase of some biologically active ones.
- Six new products were synthesized, cinchona alkaloids esters of QCI and QCD truncated, by reaction of acid chlorides with 1,2-cinchona alkaloids aminoalcohol derivatives. Newly synthesized compounds were characterized by H-NMR spectroscopy, IR, MS and elemental analysis. Synthesis yields are good.
- Three products were synthesized us with good yields, cinchona alkaloids amides of QCI and QCD truncated by reaction of acyl chlorides and 1,2-diamine QCINH2. New synthesized products were characterized H-NMR spectroscopy, IR, MS and elemental analysis.

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- We have optimized two methods of synthesis of two compounds obtained by substitution of cinchona alkaloids - cinconidina and cinconina, by establishing optimum molar ratio of reactants.
- We developed a new synthesis technology of cinconidine from cinconine by oxidation cinconinei to cinconidinone, followed by reduction with sodium borohidride, in the presence of tartaric acid. By treatment with ammonium hydroxide solution in alcoholic medium and purification, we obtained more than 99.9% purity cinconidine. Synthesis was monitored by HPLC cromatography. Product quality complies with regulatory requirements for pharmaceuticals.
- For the new product, acetylsalicylic acid ester of QCI (QCI-ASA), potential biologically active product, we have elaborated a method of analysis by liquid chromatography HPLC, establishing the optimal stationary phase and mobile phase composition, which provides a good separation.
- We validated the analytical method developed for QCI-ASA compound, assessing the accuracy, linearity, robustness, limit of detection and limit of quantification. The conducted study showed that the method of analysis developed for QCI-ASA compound is specific, robust and fast.
- The results were the subject of two articles published in magazines Study, 2011 and Revue Roumaine of Chemistry, 2012

  The results were presented as two scientific papers at national and international events.

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Selected Bibliography

1 B.T. Richard, R.B.Woodward, The Alkaloid. Chemistry and Physiology,volIII, NewYork, 1953, p2-63;

12 A.Tadensz, Alkaloids-secret of life, 2007, Elsevier;

13 L. Mink, Z. Ma, R.A. Olsen, J.N.James, D.S.Sholl, L.J.Mueller, F.Zaera ,Chiral Catalysis, 2008, 48(1-4), 120-127;

14 K.Balazsk, T.Martinek, *J.Mol.Cat*, 2007, vol 272, 265-274;

15 Encyclopedia of Physical Science and Technology, Organic Chemistry, ed.3,2002;

16 D.Kozma, C.R.C Handbook of optical resolution via diastereomere salts, 2002,CRC Press;

18 C.E.Song, Cinchona Alkaloids in Synthesis and Catalysis, 2009, Wiley-VCH;

19 A.Berkessel, H.Groger, Asymetric organocatalysis, 2005, Wiley-VCH;

21 R.P.Singh, K.Bartelsan,J.Am.Chem.Soc, 2008,vol.130, 2422-2423

29 T.G.Waddell, T.Rambalakpos,K.R.Christie,j.Org.Chem,1990,vol.55,4765-4767;

30 W.Braje, J.Frackenpohl,P.Langer,Tetrahedron,1998,vol.54, 3495-3499;

31 K.Takai, K.Nitta, K.Utimoto,J.Am.Chem.Soc,1986, vol.108, 7408-7411;

32 T.Okazoe, K.Takai, K.Utimoto,J.Am.Chem.Soc,1987,vol.109, 951-955;

33 H.M.R.Hoffmann, BvUS 5777122,1998,ClC07D;

34 H.M.R.Hoffmann, J.Frackepohl, Eur.J.org:Chem, 2004, 4293-4312;

36 C.Johansson, N.Bremeyer, L.S.V.Owen, Angew.Chem, 2006, 118, 6170,6174;

37 L.Hinterman, M.Schmitz, U.Englert, Angew.Chem.Int.Ed, 2007,46, 5164-5176;

38 A.Berkessel, M.Guixa, F.Schmidt, Chem.Eur.J,2007,13,4483-4487;

39 T.Marcelli, J.H.van Maarseveen, H.Hiemstra, Angew.Chem,2006,118,7658-7662;

40 N.Khiar, F.Alcudia, J.L.Espartero, J.Am.Chem.Soc,2002,122,7598-7600;

41 I.Fernander, N.Khiar, Chem.Rev,2003,103,3651-3655;

42 S.P.Rostaing,C.Saluzzo,R.T.Halle,Tetrahedron Asymmetry, 2001, 22, 231-235;

43 R.A.Olsen, D.Barchardl, L.Mink, J.Am.Chem:Soc,2006,128,15594-15596;

44 V.M.Maier, S.Schefzick,G.M.Lambardo, J.Am.Chem.Soc, 2002, 124, 8611-8628

45 Ho Sik, Song Ho Ohi, J. Woong Lee, Chem Commun, 2008, 1208-1210;

46 A. Erkkila, I. Majander, P.M. Pihko, Chem. Rev. 2007107, 5416 – 5470;

89 W.M.Braje, J.Holzgreff, R.Wartchow, H.M.R.Hoffmann, Angew.Chem.Int.Ed, 2000, 39,

.

2085-2087;

90 Vereinigle Chininfabriken zimmer &Co, DE 128116/1900, 129452/1915, 131723/1915;

91 H.Chen. Y.Jin, R.Jiang, catalysis Comunication, 2008, 9, 1858-1862;

109 I.Neda, T.Kaukorat, C.G.Hrib;Tetrahedron Asymmetry, 2002, 13, 1327-1330;

110 I.Neda, T.Kauborat, A.K.Fischer, Eur.J.Org.Chem, 2003, 3784-3786;

111 A.Hurtukho, S.A.Modin, P.G.Anderson, Org.Biomed.Chem, 2003 ,1, 2522-2526;

125 W.M.Braje, R.Wartchow, H.M.R.Hoffmann,Angew.Chem.Int.Ed,1999, 38(7), 2539-2543;

129 O.Schrake, H.M.Franz, R.Wartchow, H.M.R.Hoffmann, Tetrahedron, 2000, 50, 4453-4465;

130 M.H.Franz, S.Roper, R.Wartchow, H.M.R.Hoffmann, J.Org.Chem,2004,69, 2983-2991;

131 S.Roper, M.H.Franz, R.Wartchow, H.M.R.Hoffmann, J.Org.Chem, 2003, 68, 4944-4946;

132 S.Ropper, Y.Frackenpohl, O.Schroke, 2000, Org. Lett,vol2, No12, pp1661-1664;

150 R.Verpoorte, A.B.Svendsen, Chromatography of Alkaloids, part B, Gas-liquid chromatography and high performance liquid chromatography, Journal of Chromatography Library, 1984, vol. 23B, Elsevier;

151 P.B. Marhall, E.W.Rogers, Biochem J., 1945, 39(3), 258;

152 A.O.Gettler, I. Sunshine, Anal. Chem.,1951, 23(5),779;

153 D.V.McCalley, Analyst, 1990, 115, 1355-1358;

154 Z.Gong, Z.Zang, X.Yang, Analyst, 1997, 122, 283-285

155 H.Mistra, B.K.Mehta, D.C.Jain, Rec.Nat.Prod, 2008, 2(4), 107-115;

157 B.J.Kline, V.A.Turner, W.H.Barr, Analytical Chemistry,1979,51(3),440-451;

158 R.G.Achari, J.L.Baldridge, T.R.Koziol, J.Chromatogr.Sci, 1978, 16, 271-275;

166 **S. Bota,** I. Neda, L. Silaghi-Dumitrescu, Studia UBB Chemia, 2011, LVI, 3, 279-286;

167 C.P.Adams,V.V.Brantner, Drug Dev. 2006,*25*, 23–24;

168 D.J.Payne, M.N.Gwynn,D.J.Holmes,D.L.Pompliano, Drug Discovery2007, 6, 29–40;

169 F.W.Muregi, A.Ishih, Drug Development Research, 2010, 71, 20-32;

170 I.Paterson,E.A. Anderson, Science 2005, 310, 451–453;

171 D.J.Newman,G.M.Cragg, J. Nat. Prod. 2007, 70, 461–477;

172 C.H.Arnaud, Science et. Technology, 2007, 46;

173 C.M Turnbull, P. Mascario, T.A. Sheldrake, L. Lazzarato, C. Cena, A.G. Rossi,

.

J.Inflammation, 25(12), 2008, www.nvbi.nih.gov/pmc/articles/PMN2525633

174 D.C.Rees, M.Congreve, C.W. Murray, R.Carr, Nat. Rev. Drug Discovery 2004, 3, 660–672.;

175 D.S. Schellenberg, S. Abdulla, C. Ropper,C. Mol. Med, 2006, 6, 253-260;

176 A.Bigi,A.Rampa, R.Budriesi,S. Gabbi, F.Belluti, P.Ioan, E.Valoti, A. Chiarini, Bioorg. Med. Chem, 2003, 11, 1353-1361;

177 **S.Bota,** L. Silaghi-Dumitrescu, I. Neda, C. Cristea, Revue Roumaine de Chimie, 2012 , *Trimis pt. publicare*

178 H.M.R. Hoffmann, O. Schrake, Tetrahedron Asymmetry, 1998,9, 1051-1057;

179 **S. Bota,** M. Sebesan, A. Cozma, International Conference on Science, Nov. 2009;

180 R.B. Woodward, N.L.Wendel, F.J. Brutschy,J. Am.Chem.Soc, 1945, 67, 1425-1428;

181 W. E Woodward, J.Org.Chem, 1963, 28, 1431-1436;

182 **S. Bota,** International Conference on Science, Nov. 2011;

183 EMEA- ICH Q2(R1), Validation of Analytical Procedures, 1995

184 L. Roman, M.Bojita, R.Sandulescu, D.L.Muntean, Validarea metodelor analitice, 2007, Ed. Medicala