THE OPTIMIZATION OF THE CONDITIONS OF SEPARATING THE SALICYLIC ACID IN HPLC

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Abstract

This paper provide to determined the optimal conditions of separation of the salicylic acid using the cromatographic column (NUCLEOSIL C18),different compositions of the mobile phases (acetonitril, bidiestilled water, methanol, phosphoric acid) and the influence of debit on the separation by HPLC.

Key words*: salicylic acid, chromatographic column, mobile phase, HPLC*

INTRODUCTION

The salicylic acid is known for its antirheumatic action, which was discovered by Stricker in 1876, and, as a consequence, it has a very wide range of usages.

The sodium salt is used along with other synergic products in healing different rheumatic forms.

Of the worldwide production of salicylic acid, 65% is used for the production of aspirin to obtain salicylates and only 10% for other purposes.

The salicylic acid is obtained through the Kolbe-Schimtt synthesis, which consists of the carboxylation of the sodium peroxide at 125-130 degrees Celsius and at a CO_2 pressure of 7-8 at (Gocan, S., 2002).

It is presented under crystal form with (p.t) of 157-159 degrees Celsius soluble in very hot water, alcohol, ethers, glycerin and partially soluble in benzene and very soluble in chloroform when it is hot.

It is a powerful inhibitor of the pantothenic acid synthesis that is necessary to the life of microorganisms, thus manifesting its antiseptic action. His usage in the conservation of different organic substances is based on this feature. The functional derivatives of the salicylic acid have important anti-inflammatory and analog-antirheumatic properties and they are made through the reaction of either the carboxylic group or the phenolic groups (Gocan, S., 2002).

The properties of the salicylic acid are modified due to the presence of some impurities which appeared in the achieved reaction. These

impurities are the 4-hidroxi-izoftalic acid and 4-hidroxi-benzoic (European Pharmacopeea, 1997).

The admitted limits are of 0.1% for the 4-oh-izoftalic acid and of 0,05% for 4-oh-benzoic acid. The specialty literature recommends the usage of high performance liquid chromatography (HPLC) for the separation and UV detection. For this reason, HPLC with UV detection was the method used by us for the elaboration of a through and accurate method for the quantitative analysis of the impurities within the salicylic acid (European Pharmacopeea, 1997).

MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Analytical Chemistry within the University of Oradea in 2012.
For the chromatographic determination we used:

- HPLC purity methanol purchased from the Merck firm;
- HPLC purity acetonitrile purchased from the Merck firm
- Pure phosphoric acid purchased from the Merck firm
- Bi-distilled water prepared in a lab
- Analytic pure salicylic acid
- HPLC Abe & Jasco chromatograph made of:
- Pump module PU-1580
- Gradient unit LG-980-025
- Degasification module DG-980-50
- Manual injector RHEODYNE
- Detector module UV-1575
- Data processing program :BORWING 1.50
- Chromatographic column NUCLEOSIL 100 C18 5μ 10x0,46

The preparation of the testing solution

Due to the presence of 4-OH-benzoic acid and 4-OH-izoftalic acid, as impurities, we have prepared a testing solution with a content of about 0.1% of these substances. For these, we weighed 0.01g of each substance and they were introduced in proportion in a balloon of 10 ml. The substances were dissolved in methyl alcohol and it was brought at the sign with the same solvent. The solution was used for the determination having the aim to optimize the separation conditions.

Carrying out the determination

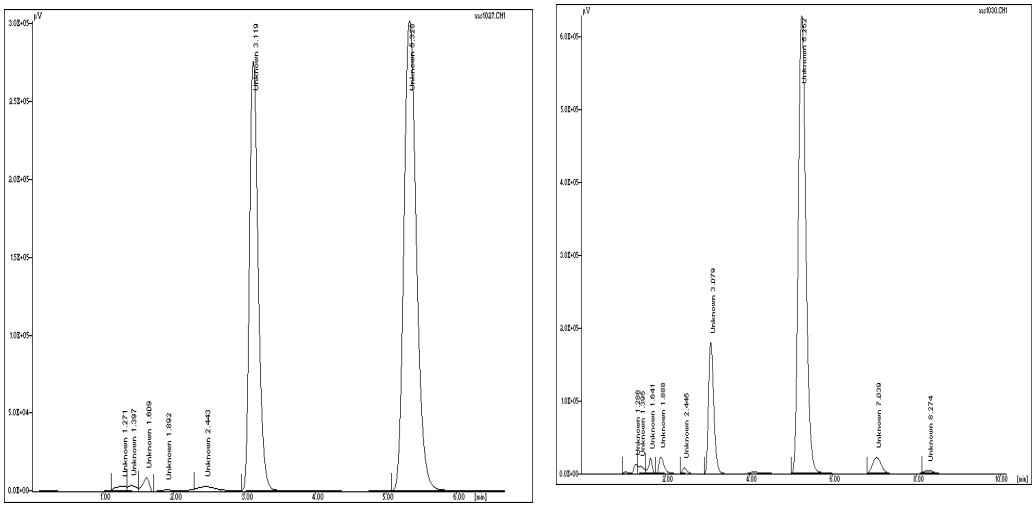
We worked with a HPLC chromatograph described at point II. The conditions at which the determination were made are:
λ = 270 nm and 237 nm
Debit: 0,5;0,7 1mL/min;

The composition of the mobile phase: acetonitrile:
$H_2O + H_3PO_4$ (300+1) = 20 : 80 ; 30 : 70
We injected 20µL of the solution that was supposed to be analyzed (tested) and the obtained chromatogram was recorded. The data were processed with a BORWING program.

RESULTS AND DISCUTIONS

A. The determination of the optimum wavelength λ:

For the determination of the optimum wavelength determinations were made keeping constant the composition of the mobile phase and debit. (Fig. 1, a and b)



a) b)

Figure1. *The chromatograms obtained for impurities by Salicylic Acid at: a)270nm; b)237 nm*

After the analysis made at the 270 nm and at 237 nm, the values obtained were as follows (table 1) for the area of the drops corresponding to the two impurities:

Table 1

The area of the drops corresponding to the two impurities

Wavelenght λ (nm)	Drops area (µV.sec)	
	Acid 4 – OH- benzoic	Acid 4 – OH- izoftalic
270 nm	2188377	3508764
237 nm	1421019	7275189

From the value of the areas and from the chromatograms (fig 1 a,b) we can see that the optimum determination is accomplished at the 270 nm wavelength .

The determination of the optimum (eluent) debit :

We worked with 3 different debits: 0,5;0,7 and 1 mL/min at a wavelength of 270 nm and the acetonitrile mobile phase : H_2 0 + H_3 PO_4 (300+1) = 20:80;

The results obtained are presented in table 2:

Table 2

Optimum (eluent) debit

Debit [mL/min]	Retention time (R_T) [min]		Resolution		Capacity	
	Acid 4 – OH - benzoic	Acid 4 -OH- izoftalic	Acid 4 – OH - benzoic	Acid 4-OH- izoftalic	Acid 4 –OH – benzoic	Acid 4-OH- izoftalic
0,5	6,169	10,633	9,13	9,46	615,93	1062,33
0,7	7,775	10,371	9,55	6,07	776,53	1036,13
1	3,119	5,328	2,35	8,77	310,87	531,80

From the results above we can see a very good separation at a debit of 0,5 mL/min but the retaining time is very high, which represents a disadvantage keeping in mind the fact that at a debit of 1mL/min the retaining time is very small, the resolution is very good and the capacity satisfactory.

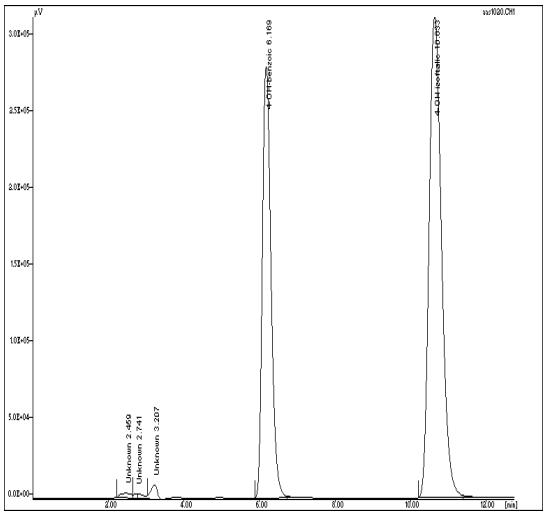


Figure 2. *The chromatogram obtained for debit obtained of the 0,5 mL/min*

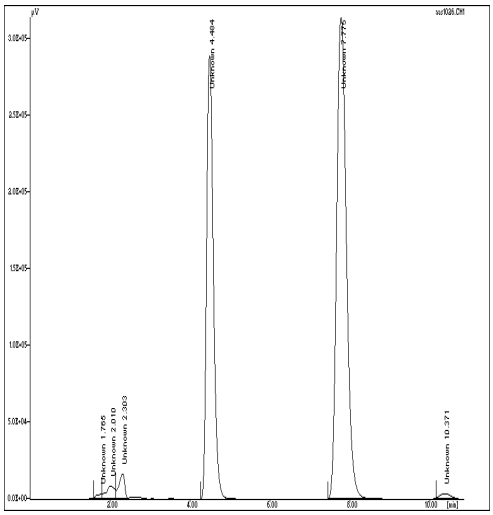


Figure 3. *The chromatogram for debit of the 0,7 mL/min*

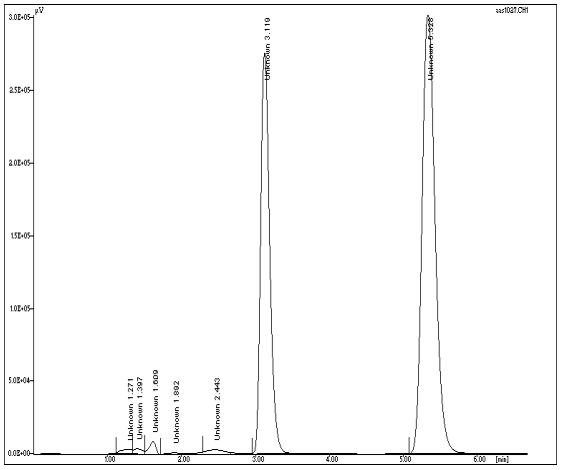


Figure 4. *The chromatogram obtained for debit of the 1 mL/min*

The fact that the debit of 1mL/min offers the best condition of separation follows from the chromatograms obtained from the 3 debit (fig. 2,3,4)

The determination of the composition of the optimum mobile phase

The separation has been done on a chromatographic column NUCLEOSIL 100 C18 5µm 10x10, 46 testing 2 compositions of the mobile phase:

Acetonitril: H_20 + H_3PO_4 (300+1) = 30:70 (Fig. 5)

Acetonitril: H_20 + H_3PO_4 (300+1) = 20:80 (Fig. 6)

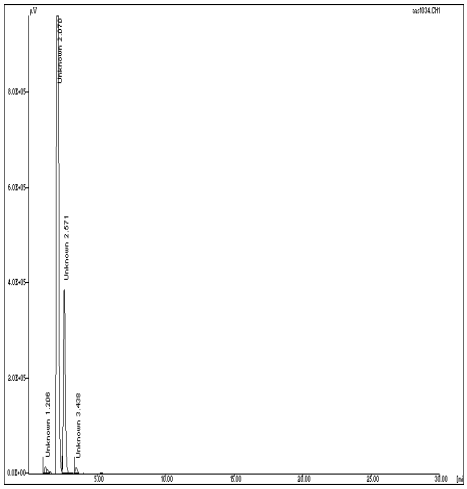


Figure5. *The chromatogram obtained at a mobile phase composition 20: 80*

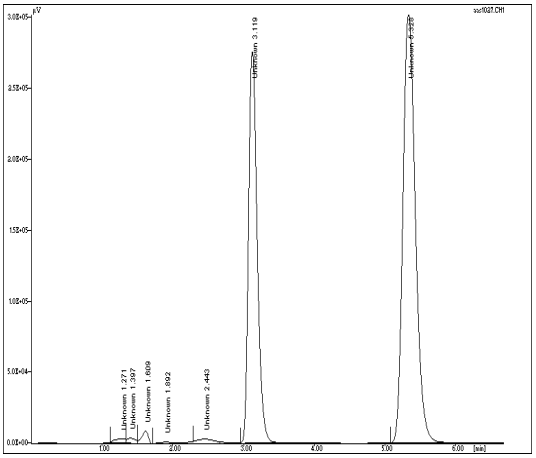


Figure6. *The chromatogram obtained at mobile phase composition 30: 70*

Table 3

Retention time, resolution and capacity values at different concentration of organic substance in the mobile phase

Mobile phase composition	Retention time (R_T) [min]		Resolution		Capacity	
	Acid 4 - OH- benzoic	Acid 4 - OH - izoftalic	Acid 4 – OH - benzoic	Acid 4 – OH - izoftalic	Acid 4 – OH - benzoic	Acid 4 - OH - izoftalic
acetonitril: H_2O+ H_3PO (300+1) = 20:80	3,119	5,328	2,35	8,77	310,87	531,80
acetonitril: H_2O+ H_3PO (300+1) = 30:70	2,571	3,438	2,23	4,41	256,13	342,80

The data from the table 3 and fig 6. indicate the fact that that a high concentration of organic substance in the mobile phase leads to unsatisfactory results, and the retaining time for a composition of the mobile phase 30:70 is very close, the resolution and the selectivity having small values .

As we can see in figure 5, the optimum composition of the mobile phase is acetonitrile: H_2O + H_3 PO_4 (300+1) = 20:80

CONCLUSIONS

From the experimental results obtained we can see that an efficient separation and a fast and accurate quantitative analysis of the salicylic acid impurities is obtained under the following circumstances:

- Detected wavelength of 270 nm;
- Mobile phase composition
- acetonitril:H_20+H_3PO_4(300+1)=20:80
- Debit (eluent) of 1mL /min.

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