Solubility and Extractability in the Pharmaceutical Sciences: A Practical Exercise with Pure Compounds

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Supplementary materials

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1. DESIGN OF EXPERIMENTS

- Selection of solvents and buffers:

The organic solvent used was chosen between ethyl acetate or diethyl ether. Although methylene chloride and chloroform are more interesting in terms of their extractability as they will position themselves at the bottom of the funnel due to their higher density making it easier for the students to recover them, they were not selected given their environmental and health hazard issues. Diethyl ether (density: 0.71: boiling point: 34.6°C) was chosen among the two choices as it is easily eliminated under reduced pressure with the rotavapor though it remains above aqueous buffers during extractions.

Regarding the aqueous phase, six different buffers are used between the range of pH 2 to 12. To facilitate the separation of both phases, each buffer is dyed. Each student performs three extractions of the compound they received according to one of both sets of pH, which are either 2,6, and 10 or 4, 8, and 12.

- Selection of dyes:

Three classical pH indicators (Bromophenol Blue, Methyl Orange and Thymol Blue) are used to dye buffers. They all have a specific color at the desired pH. With the two sets of pH, there will be three distinct colors for each pH. These compounds are easily soluble in water (buffers) and nonextractable.

- Selection of the equipment:

Gilson separatory funnels are used because they are more precise for the separation of both phases. Ideally, materials containing PTFE stopcocks are preferred as those in glass can easily get stuck. The procedure is similar to that used for the live demonstration (Liégeois et al., J. Chem. Educ. In press). During the practical session, organic phases are evaporated under reduced pressure using Buchi rotavapor apparatus.

2. MATERIALS AND REAGENTS

- Individual materials (for two students or a table):

- One Gilson separatory funnel (250 mL) with a PTFE stopcock and a plastic stopper

- One evaporating flask (250 mL)

- One flask ring support

- Two graduated cylinders (100 mL)

- Two glass funnels

- One filter funnel support

- One support ring (with or without bosshead)

- One stand base

- One beaker low form (150 mL)

- Two Erlenmeyer flasks narrow mouth (100 and 150 mL)

- pH indicator paper roll

- glass stick

- Common materials:

- Analytical balances Mettler ME 2002 and 3002 (n = 8)

- Rotavapor R-100 Buchi with water batch and vacuum pump (n = 6)

- Sartorius paper folded filters qualitative grade 1288

- Reagents:

Six buffers at pH 2, 4, 6, 8, 10, and 12 (see below for preparation)

Diethyl ether (i.e. Thermofischer D/2450/17)

Magnesium sulfate, anhydrous (i.e. ThermoScientific 196850010)

3. PREPARATION OF BUFFERS

- Compounds:

Glycine, citric acid (from VWR)

Sodium hydroxide, monopotassium phosphate (from Fischer Scientific)

Disodium phosphate dihydrate, Methyl Orange indicator, Thymol Blue indicator (Merck)

Bromophenol Blue indicator, hydrochloric acid fuming 37 % (ACROS)

- Dye solutions:

Solution 1:

An ethanolic stock solution of Methyl Orange was prepared in water at 2.5 g/L

Solution 2:

An ethanolic stock solution of Thymol Blue at 1 g/L was prepared.

Solution 3:

A stock ethanolic solution of Bromophenol Blue at 1 g/L was prepared.

- Buffered solutions:

Each buffer is dyed (Figure S1) to easily visualize more the biphasic mixture.

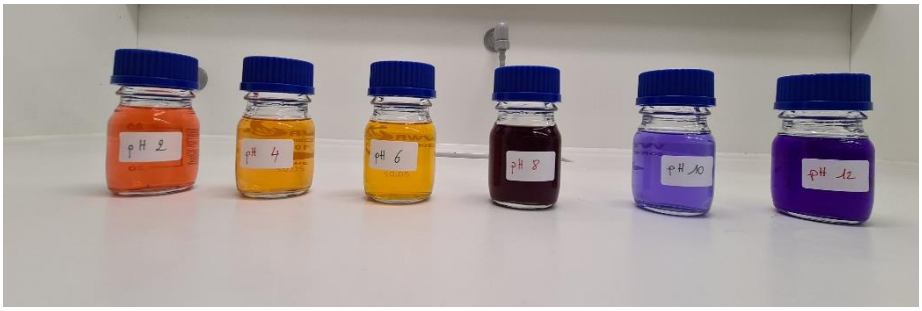


Figure S1: Buffers for extractability experiments (from pH 2 to pH 12, from left to right)

- **For 10 liters of buffer at pH = 2:**

38.06 g of glycine and 29.63 g of NaCl were dissolved in 5,07 L of deionized water. After solubilization, 4,93 L of a solution of 0,1 M HCl were added.

20 mL of Solution 1 were added to color the buffer.

- **For 10 liters of buffer at pH = 4:**

115.79 g of citric acid were dissolved in 4.41 L of deionized water and added to 1.1 L of a 1 M NaOH solution. After solubilization, 4,49 L of a solution of 0,1 M HCl were added.

20 mL of Solution 1 were added to color the buffer.

- **For 10 liters of buffer at pH = 6:**

80.66 g of monopotassium phosphate were dissolved in 8.89 L of deionized water.

13.18 g of disodium phosphate dihydrate were dissolved in 1.11 L of deionized water.

The two solutions are mixed after solubilization.

10 mL of Solution 2 were added to color the buffer.

- **For 10 liters of buffer at pH = 8:**

3.36 g of monopotassium phosphate were dissolved in 0.37 L of deionized water.

114.31 g of disodium phosphate dihydrate were dissolved in 9.63 L of deionized water.

The two solutions are mixed after solubilization.

10 mL of Solution 2 were added to color the buffer.

- **For 10 liters of buffer at pH = 10:**

46.92 g of glycine and 36.53 g of NaCl were dissolved in 6.25 L of deionized water. After solubilization 3.75 L of a solution of 0,1 M NaOH were added.

10 mL of Solution 3 were added to color the buffer.

- **For 10 liters of buffer at pH = 12:**

34.53 g of glycine and 26.88 g of NaCl were dissolved in 4.6 L of deionized water. After solubilization 5.4 L of a solution of 0,1 M NaOH were added.

10 mL of Solution 3 were added to color the buffer.

After preparation the pH of each buffer is controlled. The pH stability is checked over the time and they are stable for several weeks.

4. PREPARATION OF SAMPLES

The samples are weighed in a vial (1 to 1.3 g.). Each sample has a code. Currently, the codes are as follows: A for benzoic acid, B for sodium benzoate, ….

Once the laboratory session is over, the assistant checks the amount of substance remaining in the vial for the evaluation of the report.

5. HAZARDS and SAFETY

A laboratory coat and safety glasses are mandatory to be able to enter the lab.

Students, before any lab session, follow a mandatory “safety seminar” to show locations of the eyewash station, safety showers, extinguishers, and various local safety information. During this seminar, students were made aware of the procedure to follow in case of an accident, the meaning of different pictograms, and waste management. All of this information can be found in the lab manual and students are tested to assess their knowledge about the subject.

In case a mistake is made by a student, the assistant, student assistants and the technician are aware of safety procedures to enforce and will exercise caution throughout the laboratory session. Diphoterine® sprays are available in the lab for any chemical injury.

- Chemicals:

- **Benzoic acid**

Benzoic acid can cause skin irritation, eye damage, and organs damage through prolonged or repeated exposure.

- **Salicylic acid**

Salicylic acid is harmful if swallowed, can cause serious eye damage and is suspected of damaging the unborn child.

- ***O*-Acetylsalicylic acid**

O-acetylsalicylic acid is harmful if swallowed, causes eye, skin, and respiratory tract irritation. Hygroscopic (absorbs moisture from the air)

- **Sodium benzoate**

Sodium benzoate can cause eye irritation.

- **Sodium salicylate**

Sodium salicylate is harmful if swallowed and can cause serious eye irritation.

- **Menthol**

Menthol causes skin irritation and serious eye irritation. It may also cause respiratory irritation.

- **Vanillin**

Vanillin causes serious eye irritation

- ***para*-Hydroxybenzaldehyde**

para-Hydroxybenzaldehyde causes skin irritation and serious eye irritation.

- **Thymol**

Thymol is harmful if swallowed and causes severe skin burns and eye damage. It may also cause respiratory irritation.

- ***para*-Nitrobenzoic acid**

para-Nitrobenzoic acid is harmful if swallowed and causes skin irritation and serious eye irritation. It is suspected of causing genetic defects, cancer and damaging fertility or the unborn child.

- **Prilocaine**

Prilocaine is harmful if swallowed and causes skin irritation and serious eye irritation.

- **Quinidine sulfate**

Quinidine sulfate is harmful if swallowed.

- ***para*-Aminobenzoic acid**

para-Aminobenzoic acid may form combustible dust concentrations in air.

6. PROTOCOLS

Before introducing these experiments as a practical session, a few undergraduate students and student assistants have tested and optimized the procedure. Different parameters and different compounds were assessed.

- Solubility:

A few drops of water or the organic solvent are added to 10-20 mg of their compound. If the compound is solubilized the information is reported in the laboratory report. If not, few more drops of water or the organic solvent are added to see if the compound is solubilized or not. For pH measurement, few mg of compound are put on a piece of pH indicator paper and then water is added in order to have a high concentration of compound. The pH value is then reported in the laboratory report.

- Extractability:

Students have previously been taught how to manipulate a separatory funnel following a detailed video and have had the occasion to practice during a previous synthesis done in the laboratory.

Each student receives a vial with ~ 1 g of the compound to be tested.

The separatory funnel is used and installed in the hood. 100 mL of appropriate buffer is poured in the separatory funnel followed by 50 mL of diethyl ether. 250 mg of the compound is weighed precisely and is directly poured into the separatory funnel. A plastic stopper is placed and the mixture is vigorously shaken for 5 seconds. No trace of the compound should be visible at this step. If some particles remain in suspension, an additional shaking is necessary.

The overpressure in the separatory funnel is removed carefully and the mixture is left to settle until both phases are clearly separated. The buffer phase is carefully recovered in a beaker and conserved for a second extraction, while the organic phase is kept in a dry Erlenmeyer. The second extraction is done in the same manner as previously described. Both organic phases are then assembled in the Erlenmeyer and dried with 500 mg - 1 g of

anhydrous $MgSO_4$. During this step, the Erlenmeyer is covered with a Petri dish or a crystallizing dish. The suspension is stirred for approximately five minutes. Thereafter, the organic solution is filtered using a paper folded filter to remove the mineral and then collected in a tare flask. The erlenmeyer is rinced 2 times with 10 mL of fresh diethyl ether and the solvent is also collected in the tare flask. Finally, supervisors help students eliminate diethyl ether under reduced pressure and the flask carefully dried is then weighed. The same procedure is then reproduced with two other buffers.

The difference in weight before and after evaporation is reported in the laboratory report which indicates whether the product is extracted totally, partially or not at all. The value is then used to get the percentage of extracted product.

Each student will represent the three values obtained in a graph. Thereafter, the data is gathered with those of the rest of the class in a single graph (Figure S2).

7. ORGANIZATION (4-hour session)

The class is divided into groups at the beginning of the academic year. The laboratory has 22 tables and hoods. It is divided into two parts. Thus, a maximum of 44 students is present for a session.

Two students are working at each table. Extractions and all the manipulations are performed in the hood.

Each student receives a sample and has three extractions to be done with this compound at three different pH values (pH 2, 6, and 10 or pH 4, 8, and 12). Other pH values have been tested and the data are also added in the corresponding table and graph when needed.

During each session, the assistant is helped by two student assistants, from third-year Bachelors to second-year Masters in Pharmaceutical Sciences, and one technician. Student assistants have experience in practical sessions but a training is organized so they may learn how to manipulate rotavapors (three machines in each part of the laboratory).

8. RESULTS

The experiments were performed during three academic years as detailed in Table S1.

Table S1: Distribution of students during the last three academic years

	Academic year		
	2021-2022	2022-2023	2023-2024
Number of groups	6	6	6
Number of students per group	19-20	15-16	15-16
Number of students in total	117	91	93

During the laboratory session, students are asked to indicate their own results in a global graph. A copy of one of them is added below (Figure S2).

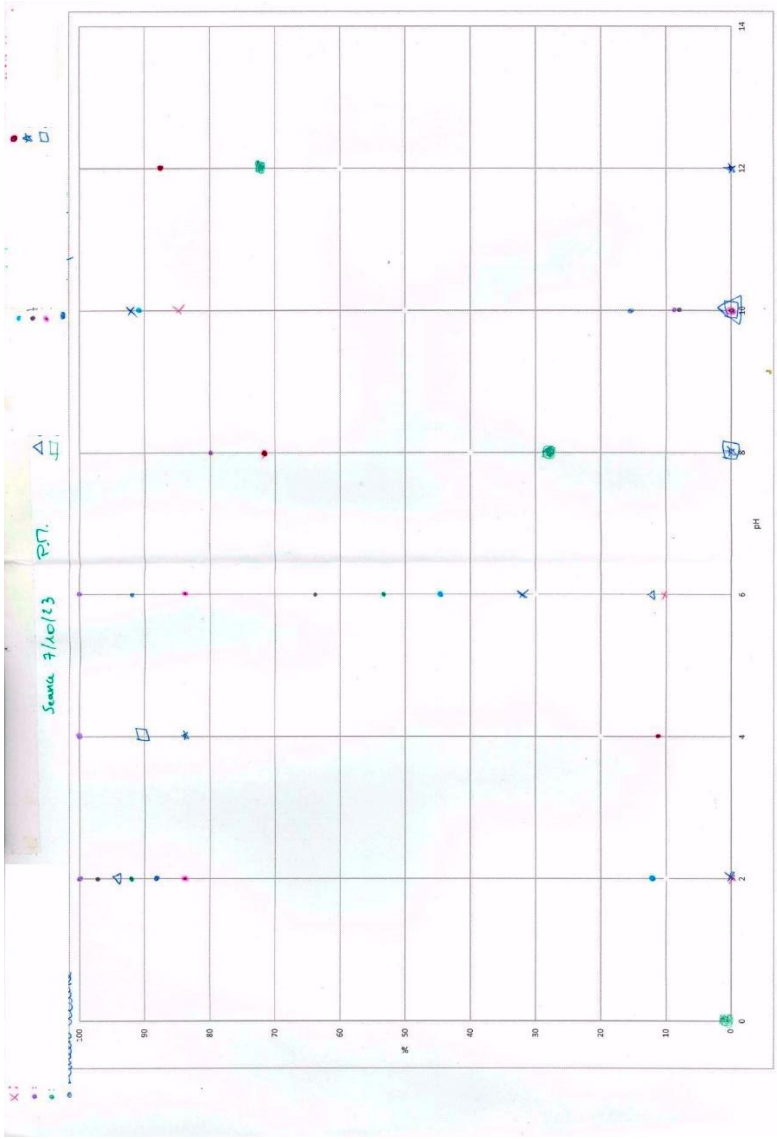


Figure S2: A copy of the table installed during the laboratory in which students indicate their own results.

In table S2, the number of data collected for each compound with the percentage of success and the percentage of outliers.

Table S2: Number of data collected for each compound with the percentage of success and the percentage of outliers.

	Values (n=)	Outliers (n=)	% success	% outliers
Benzoic acid	43	6	86,0	14,0
Sodium benzoate	36	4	88,9	11,1
Salicylic acid	33	3	90,9	9,1
Sodium salicylate	33	5	84,8	15,2
O-Acetylsalicylic acid	30	3	90,0	10,0
p-Nitrobenzoic acid	45	6	86,7	13,3
p-Aminobenzoic acid	45	5	88,9	11,1
Vanillin	48	8	83,3	16,7
p-Hydroxybenzaldehyde	60	6	90,0	10,0
Thymol	22	2	90,9	9,1
Menthol	30	3	90,0	10,0
Prilocaine	54	14	74,1	25,9
Quinidine sulfate	45	11	75,6	24,4

All the results for all compounds tested are represented below. All data are expressed in percentage of starting material. The first table contains the crude data of each compound. Outliers are indicated in red and exceeded the mean by 15 %. The second table contains results excluding outliers. The graph is made with the corrected values from the second table.

- Data obtained for benzoic acid:

a)

pH	2	4	6	8	10	12
	96	92	72	0,8	4	0
	96	96	72	4	4	0
	100	100	56	12	0	0
	88	100	84	16	0	0
	86,7	20	76	16	28	4
	92,3	16	12	4	0	0
	96	100	0	4	0	0
	84	88	84	52	12	0
	100	92	56	16	48	0
	96	100	76		4	0
	96	80	68			0
						4
Mean	93,7	80,4	59,6	13,9	10,0	0,7
SD	5,3	31,5	28,2	15,5	15,9	1,6

b)

pH	2	4	6	8	10	12
	96	92	72	0,8	4	0
	96	96	72	4	4	0
	100	100	56	12	0	0
	88	100	76	16	0	0
	86,67	100	56	16	0	4
	92,3	88	76	4	0	0
	96	92	68	4	12	0
	84	100		16	4	0
	100	80				0
	96					0
	96					0
						4
Mean	93,7	94,2	68,0	9,1	3,0	0,7
SD	5,3	7,0	8,6	6,5	4,1	1,6

Table S3. Compilation of results obtained by students whose sample contained benzoic acid: a) crude value b) values without outliers

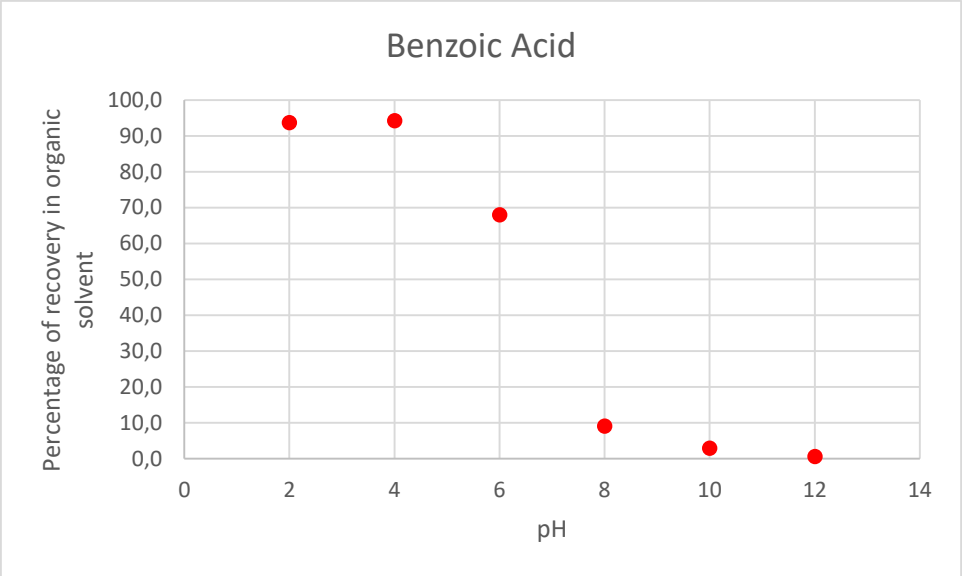


Figure S3. Extractability curve obtained by students whose sample contained benzoic acid. (without outliers)

- Data obtained for sodium benzoate:

a)

pH	2	4	6	8	10	12
	84	100	28	28	0	24
	68	40	32	8	0	0
	92	80	25	8	4	8
	84		38,5		12	4
	68		20		0	0
	88,5		28		0	4
Mean	80,7	73,3	28,6	14,7	2,7	6,7
SD	10,3	30,6	6,3	11,5	4,8	9,0

b)

pH	2	4	6	8	10	12
	84	100	28	8	0	0
	68	80	32	8	0	8
	92		25		4	4
	84		38,5		12	0
	68		20		0	4
	88,5		28		0	
Mean	80,7	90,0	28,6	8,0	2,7	3,2
SD	10,3	14,1	6,3	0,0	4,8	3,3

Table S4. Compilation of results obtained by students whose sample contained sodium benzoate: a) crude value b) values without outliers

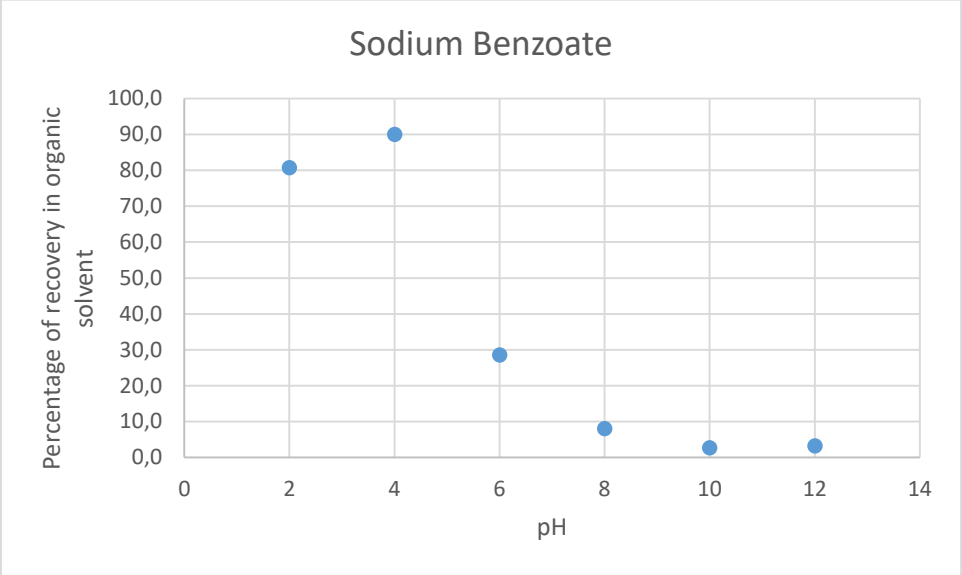


Figure S4. Extractability curve obtained by students whose sample contained sodium benzoate. (without outliers)

- Data obtained for salicylic acid:

a)

pH	2	4	6	8	10	12
	100	95,8	32	15,4	0	4
	80	100	68	4	4	4
	92	96	72	8	0	0
	88,5	100,0	40,0	0,0	3,9	4,2
	76,9	92	76	25	8	11
	96	57	64	0	4	0
	83,3	72,0	64,0	16,0	0,0	8,0
	88,5	100,0	57,7	0,0	3,9	0,0
	96	92	64	0	8	0
	100	88	60	12	12	0
	100	72	68	4	0	4
Mean	91,0	87,7	60,5	7,7	4,0	3,2
SD	8,3	14,4	13,3	8,4	4,0	3,7

b)

pH	2	4	6	8	10	12
	100	95,8	68	15,4	0	4
	80	100	72	4	4	4
	92	96	76	8	0	0
	88,5	100,0	64,0	0,0	3,9	4,2
	76,9	92,0	64,0	0,0	8,0	11,0
	96,0	72,0	57,7	16,0	4,0	0,0
	83,3	100	64	0	0	8
	88,5	92,0	60,0	0,0	3,9	0,0
	96	88	68	12	8	0
	100	72		4	12	0
	100				0	4
Mean	91,0	90,8	66,0	5,9	4,0	3,2
SD	8,3	10,7	5,7	6,5	4,0	3,7

Table S5. Compilation of results obtained by students whose sample contained salicylic acid: a) crude value b) values without outliers

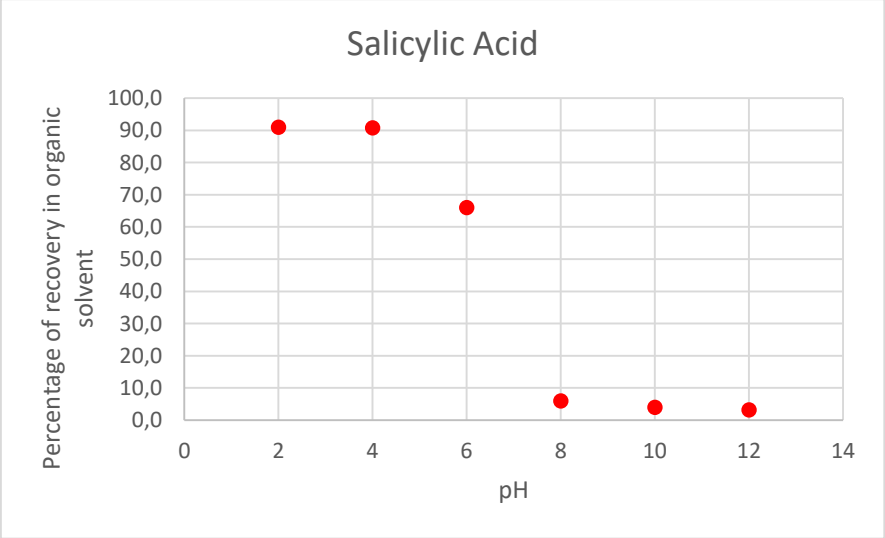


Figure S5. Extractability curve obtained by students whose sample contained salicylic acid. (without outliers)

- Data obtained for sodium salicylate:

a)

pH	2	4	6	8	10	12
	92	92	12	16	4	4
	52,0	92,0	23,1	4,0	3,7	0,0
	88,0	92,3	19,2	24,0	4,0	20,0
	100,0	68,0	8,0	8,0	4,0	8,0
	92,0	80,0	4,0	11,5	0,0	4,0
	96,0	88,0	12,0	30,0	4,0	20,5
	99,8	64,0	12,0	0,0	0,0	0,0
	96,0	87,5	0,0	11,5	0,0	0,0
	73,0	44,0	32,0	0,0	0,0	0,0
	76,0	73,1	0,0	3,9	0,0	0,0
	88		40		0	
Mean	86,6	78,1	14,8	10,9	1,8	5,7
SD	14,4	15,9	12,8	10,0	2,1	8,1

b)

pH	2	4	6	8	10	12
	92	92	12	16	4	4
	88,0	92,0	23,1	4,0	3,7	0,0
	100,0	92,3	19,2	24,0	4,0	8,0
	92	80	8	8	4	4
	96	88	4	11,5	0	0
	99,8	87,5	12,0	0,0	4,0	0,0
	96,0	73,1	12,0	11,5	0,0	0,0
	73,0		0,0	0,0	0,0	0,0
	76,0		0,0	3,9	0,0	
	88				0	
					0	
Mean	90,1	86,4	10,0	8,8	1,8	2,0
SD	9,2	7,3	8,0	7,9	2,1	3,0

Table S6. Compilation of results obtained by students whose sample contained sodium salicylate: a) crude value b) values without outliers

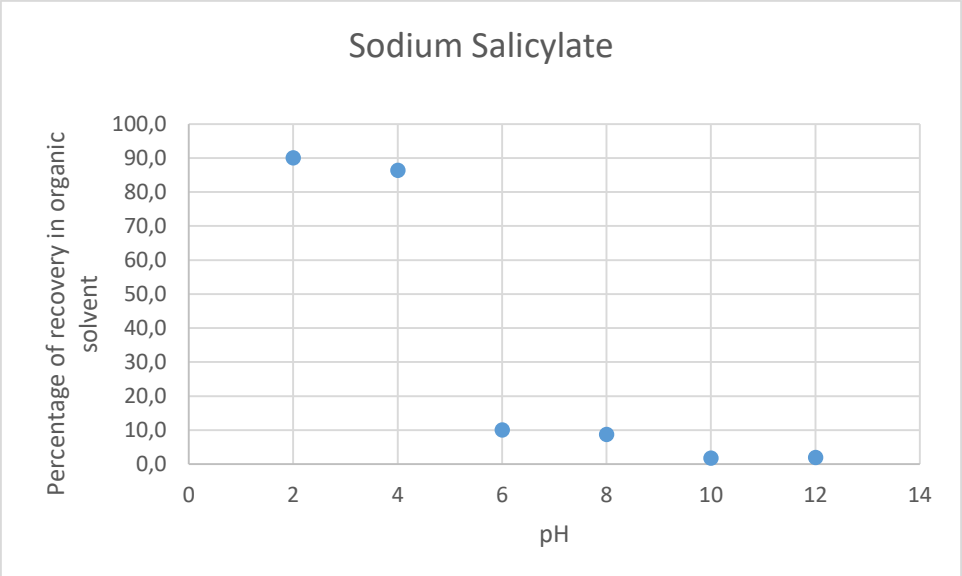


Figure S6. Extractability curve obtained by students whose sample contained sodium salicylate. (without outliers)

- Data obtained for *O*-acetylsalicylic acid:

a)

pH	2	4	6	8	10	12
	80	96	96	0	10	0
	92,3	80	63	0	0	0
	88	84	56	4	0	4
	76	92	80	0	8	0
	80,8	88,0	46,0	20,0	4,0	8,0
	88,0	73,1	80,0	0,0	0,0	0,0
	92,3	85,7	40,0	7,4	0,0	0,0
	92,0	68,0	56,0	0,0	3,9	0,0
	92	80	52	4	0	4
	88		12		0	
	84		40		4	
Mean	86,7	83,0	56,5	3,9	2,7	1,8
SD	5,7	8,8	23,2	6,6	3,6	2,9

b)

pH	2	4	6	8	10	12
	80	96	63	0	10	0
	92,3	80	56	0	0	0
	88	84	80	4	0	4
	76	92	80	0	8	0
	80,8	88,0	56,0	0,0	4,0	8
	88,0	73,1	52,0	7,4	0,0	0
	92,3	85,7		0,0	0,0	0
	92,0	68,0		4,0	3,9	0
	92	80			0	4
	88				0	
	84				4	
Mean	86,7	83,0	64,5	1,9	2,7	1,8
SD	5,7	8,8	12,5	2,9	3,6	2,9

Table S7. Compilation of results obtained by students whose sample contained O-acetylsalicylic acid: a) crude value b) values without outliers

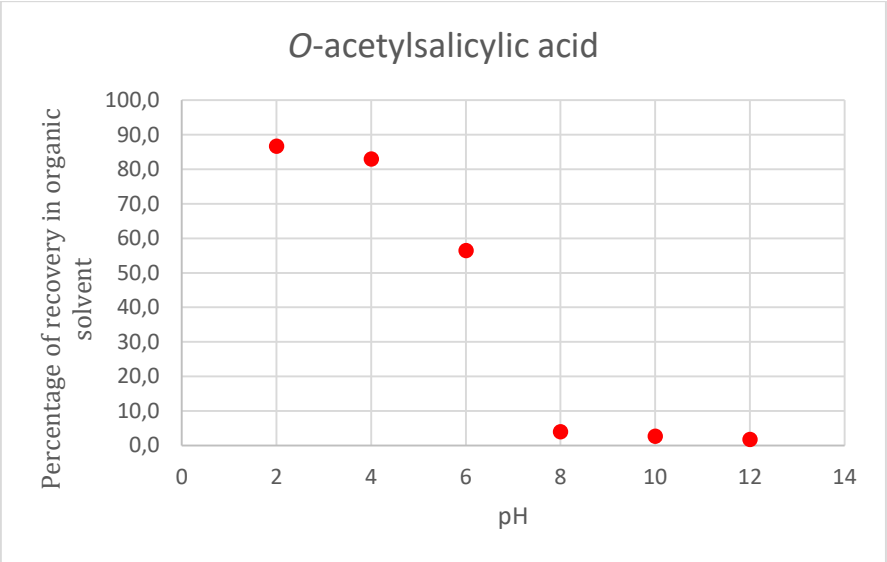


Figure S7. Extractability curve obtained by students whose sample contained *O*-acetylsalicylic acid. (without outliers)

- Data obtained for *para*-nitrobenzoic acid:

a)

pH	2	4	6	8	10	12
	92,6	84,0	92,0	3,9	0,0	3,9
	28,0	80,0	6,4	20,0	1,5	16,0
	81,5	88,0	73,1	8,5	0,0	0,0
	96,2	96,2	73,1	15,4	7,7	20,0
	84,0	96,0	32,0	3,8	4,0	4,2
	96,0	84,6	96,0	0,0	24,0	0,0
	88,0	92,9	48,0	8,0	8,0	0,0
	92,3	84,0	53,9	12,0	0,0	0,0
	96,3	84,0	66,7	0,0	0,0	0,0
	92,0	81,0	62,0	0,0	3,8	0,0
	100	73,7	64	5	0	3
Mean	86,1	85,8	60,6	7,0	4,5	4,3
SD	20,0	6,9	25,6	6,6	7,2	7,0

b)

pH	2	4	6	8	10	12
	92,6	84,0	73,1	3,9	0,0	3,9
	81,5	80,0	73,1	20,0	1,5	16,0
	96,2	88,0	48,0	8,5	0,0	0,0
	84,0	96,2	53,9	15,4	7,7	4,2
	96,0	96,0	66,7	3,8	4,0	0,0
	88,0	84,6	62,0	0,0	8,0	0,0
	92,3	92,9	64,0	8,0	0,0	0,0
	96,3	84,0		12,0	0,0	0,0
	92,0	84,0		0,0	3,8	0,0
	100	81		0	0	3
		73,7		5		
Mean	91,9	85,8	63,0	7,0	2,5	2,7
SD	5,8	6,9	9,4	6,6	3,2	5,0

Table S8. Compilation of results obtained by students whose sample contained *para*-nitrobenzoic acid: a) crude value b) values without outliers

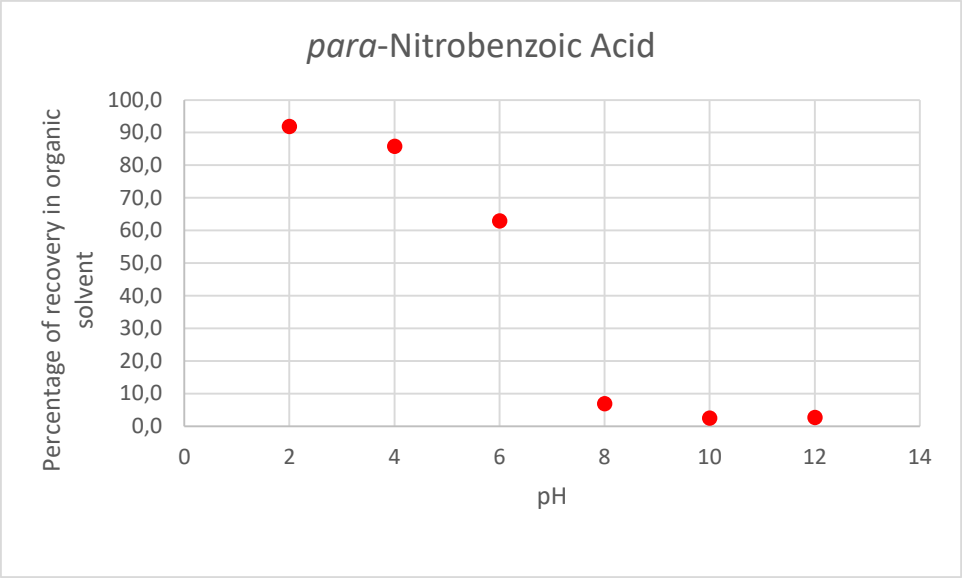


Figure S8. Extractability curve obtained by students whose sample contained *para*-nitrobenzoic acid. (without outliers)

- Data obtained for *para*-aminobenzoic acid:

a)

pH	2	4	6	8	10	12
	56,0	92,0	60,0	12,0	12,0	4,0
	48,0	100,0	60,0	12,0	0,0	0,0
	57,7	82,6	56,0	4,0	8,0	0,0
	52,0	65,4	48,0	8,0	0,0	24,0
	27,0	80,0	52,0	8,0	7,7	0,0
	44,0	92,0	73,0	22,0	0,0	4,0
	33,0	76,0	36,0	11,5	4,2	0,0
	26,1	80,0	56,0	0,0	8,0	0,0
	64,0	80,0	72,0	0,0	0,0	0,0
	64,0	60,0	60,0	4,0	0,0	0,0
Mean	47,2	80,8	57,3	8,2	4,0	3,2
SD	14,3	12,1	10,8	6,7	4,6	7,5

b)

pH	2	4	6	8	10	12
	56	92	60	12	12	4
	48	82,6	60	12	0	0
	57,7	80	56	4	8	0
	52	92	48	8	0	0
	44	76	52	8	7,7	4
	33	80	73	22	0	0
	64,0	80,0	56,0	11,5	4,2	0,0
	64		72	0	8	0
			60	0	0	0
				4	0	
Mean	52,3	83,2	59,7	8,2	4,0	0,9
SD	10,5	6,3	8,3	6,7	4,6	1,8

Table S9. Compilation of results obtained by students whose sample contained *para*-aminobenzoic acid: a) crude value b) values without outliers

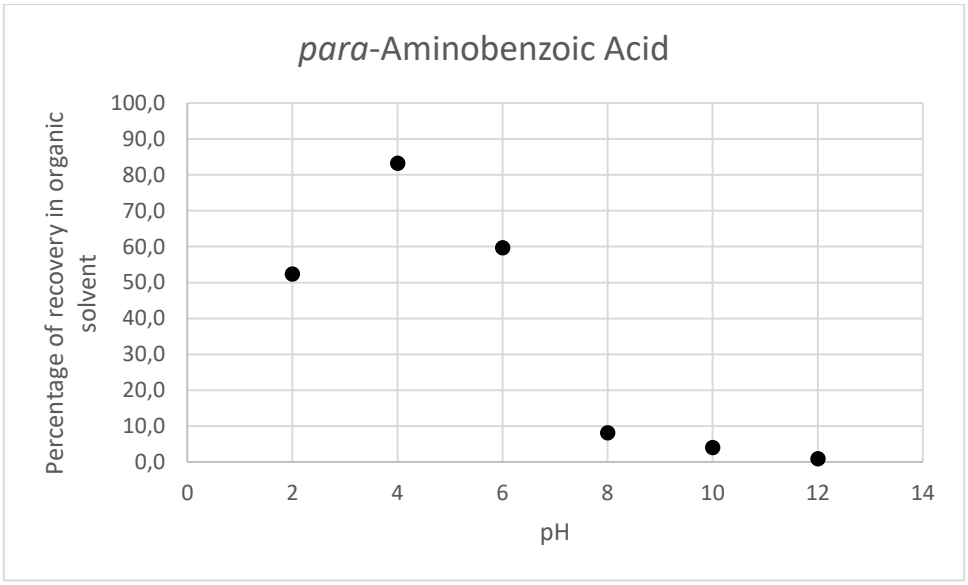


Figure S9. Extractability curve obtained by students whose sample contained *para*-aminobenzoic acid. (without outliers)

- Data obtained for vanillin:

a)

pH	2	4	6	8	10	12
	84	96,3	96	76	12	72
	96	96	96	68	12	56
	92	88	88	64	12	3,8
	86,4	80	84	64	15,4	3,6
	100	84,6	76	96,2	16	0
	88	92,3	85	80,8	4	0
	84		80		56	0
	92		64		0	8
	88		92,3		16	4
	96		100		8	
Mean	90,6	89,5	86,1	74,8	15,1	16,4
SD	5,5	6,5	10,9	12,4	15,3	27,4

b)

pH	2	4	6	8	10	12
	84	96,3	96	76	12	3,8
	96	96	96	68	12	3,6
	92	88	88	64	12	0
	86,4	80	84	64	15,4	0
	100	84,6	76	80,8	16	0
	88	92,3	85		4	8
	84		80		16	4
	92		92,3		8	
	88		100			
	96					
Mean	90,6	89,5	88,6	70,6	11,9	2,8
SD	5,5	6,5	8,1	7,5	4,2	3,0

Table S10. Compilation of results obtained by students whose sample contained vanillin: a) crude value b) values without outliers

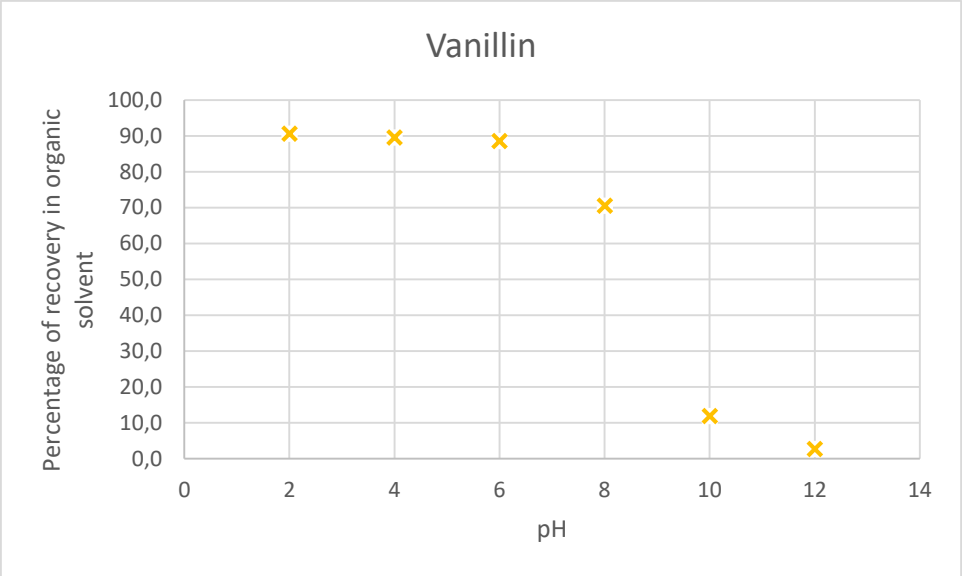


Figure S10. Extractability curve obtained by students whose sample contained vanillin. (without outliers)

- Data obtained for *para*-hydroxybenzaldehyde:

a)

pH	2	4	6	8	10	12
	95,8	84,6	91,7	73,1	24,0	12,0
	100,0	100,0	77,0	75,0	27,0	8,3
	88,0	100,0	100,0	84,0	20,0	16,0
	92,0	96,0	100,0	84,0	11,0	4,0
	88,0	68,0	100,0	76,0	64,0	72,0
	100,0	84,0	100,0	73,1	20,0	3,7
	84,0	100,0	92,0	69,2	20,0	16,0
	96,2	92,0	84,6	88,0	52,0	0,0
	84,0	74,0	92,3	92,0	15,4	20,0
	88,0	88,0	96,2	63,0	20,0	3,5
Mean	91,6	88,7	93,4	77,7	27,3	15,5
SD	6,1	11,2	7,7	9,0	17,0	20,9

b)

pH	2	4	6	8	10	12
	95,8	84,6	91,7	73,1	24,0	12,0
	100,0	100,0	100,0	75,0	27,0	8,3
	88,0	100,0	100,0	84,0	20,0	16,0
	92,0	96,0	100,0	84,0	11,0	4,0
	88,0	84,0	100,0	76,0	20,0	3,7
	100,0	100,0	92,0	73,1	20,0	16,0
	84,0	92,0	84,6	69,2	15,4	0,0
	96,2	88,0	92,3	88,0	20,0	20,0
	84,0		96,2	92,0		3,5
	88,0			63,0		
Mean	91,6	93,1	95,2	77,7	19,7	9,3
SD	6,1	6,9	5,4	9,0	4,9	7,0

Table S11. Compilation of results obtained by students whose sample contained *para*-hydroxybenzaldehyde: a) crude value b) values without outliers

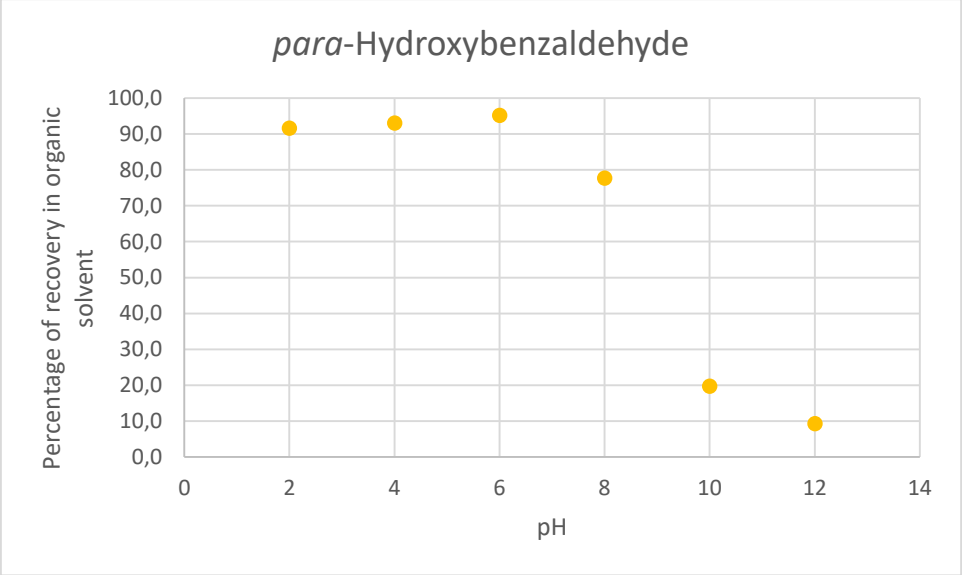


Figure S11. Extractability curve obtained by students whose sample contained *para*-hydroxybenzaldehyde. (without outliers)

- Data obtained for thymol:

For this compound, we need to add more values at higher pH to visualize the deprotonation of the phenol group. Data are in bold.

a)

pH	2	4	6	8	10	12	**13**	**14**
	112	100	92	88	84	96,2	**68**	**67,9**
	100	100	96	80	100	84,0	**75**	**69,2**
	81	92	96	96	100	92,0		
	92	100	92	96	100	100,0		
	83,3		92		100			
	92		80		100			
	92		20		96			
	100		96		100			
	88		96		100			
Mean	93,4	98,0	84,4	90,0	97,8	93,0	**71,5**	**68,5**
SD	9,5	4,0	24,7	7,7	5,3	6,9	**4,9**	**1,0**

b)

pH	2	4	6	8	10	12	**13**	**14**
	100	100	92	88	84	96,2	**68**	**67,9**
	81	100	96	80	100	84	**75**	**69,2**
	92	92	96	96	100	92		
	83,3	100	92	96	100	100		
	92		92		100			
	92		80		100			
	100		96		96			
	88		96		100			
					100			
Mean	91,0	98,0	92,5	90,0	97,8	93,0	**71,5**	**68,5**
SD	6,9	4,0	5,4	7,7	5,3	6,9	**4,9**	**1,0**

Table S12. Compilation of results obtained by students whose sample contained thymol: a) crude value b) values without outliers

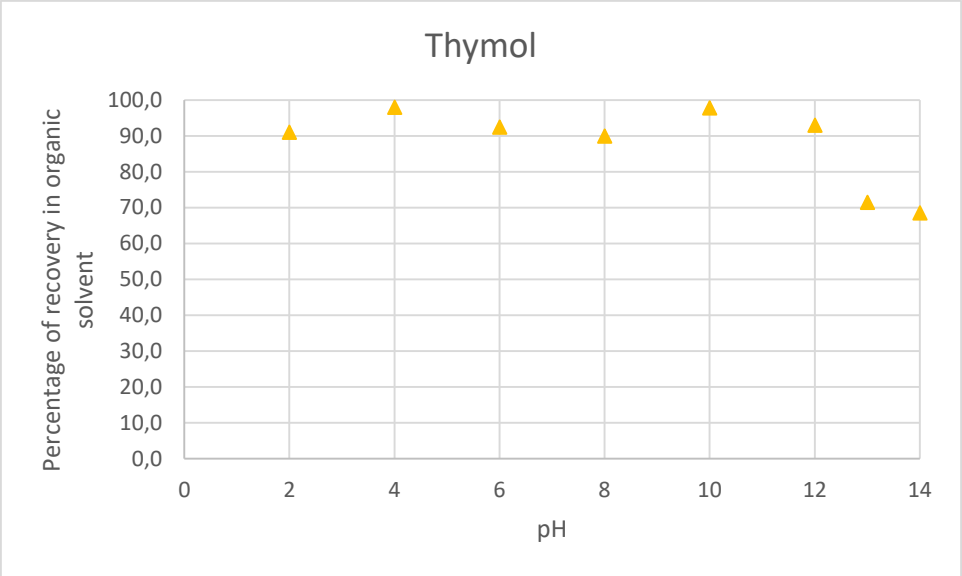


Figure S12. Extractability curve obtained by students whose sample contained thymol. (without outliers)

- Data obtained for menthol:

a)

pH	2	4	6	8	10	12
	100	80,8	92	96	100	96
	100	92	72	92,3	64	92,3
	88	100	92	92	88	100
	84	91,7	84	96	73	100
	100	92,3	96	84	80	95,8
	100	100	92	95,8	92	100
	92,3	84	96,2	80	96	88
	100		100	80		8
				32		
Mean	95,5	91,5	90,5	83,1	84,7	85,0
SD	6,5	7,3	8,8	20,3	13,0	31,4

b)

pH	2	4	6	8	10	12
	100	80,8	92	96	100	96
	100	92	92	92,3	88	92,3
	88	100	84	92	80	100
	84	91,7	96	96	92	100
	100	92,3	92	84	96	95,8
	100	100	96,2	95,8		100
	92,3	84	100	80		88
	100			80		
Mean	95,5	91,5	93,1	89,5	91,2	96,0
SD	6,5	7,3	5,0	7,1	7,7	4,6

Table S13. Compilation of results obtained by students whose sample contained menthol: a) crude value b) values without outliers

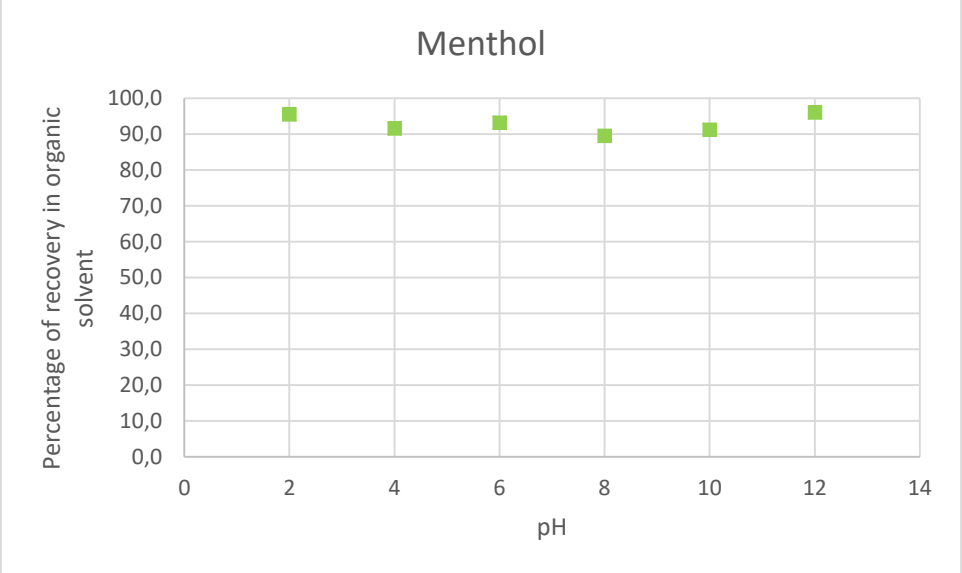


Figure S13. Extractability curve obtained by students whose sample contained menthol. (without outliers)

- Data obtained for prilocaine:

a)

pH	2	4	6	8	10	12
	4,2	20,8	41,7	100,0	96,0	84,0
	8,0	4,0	52,0	60,0	72,0	92,6
	26,9	7,1	48,0	12,5	88,0	104,0
	4,0	0,0	46,0	96,2	92,0	84,6
	20,0	8,0	20,0	3,6	80,0	4,7
	8,0	20,0	60,0	80,0	76,0	92,0
	0,0	12,0	52,0	88,0	100,0	91,7
	0,0	0,0	44,0	88,0	92,0	74,1
	0,0	16,0	21,6	96,0	21,6	80,0
	12	12	44	72	92	88
	0	0	32	88,5	92	100
	0		44		84	
	0		68		88	
Mean	6,4	9,1	44,1	71,3	82,6	81,4
SD	8,7	7,7	13,6	33,4	20,0	26,8

b)

pH	2	4	6	8	10	12
	4,2	4,0	41,7	100,0	96,0	84,0
	8,0	7,1	52,0	96,2	88,0	92,6
	4,0	0,0	48,0	80,0	92,0	84,6
	8,0	8,0	46,0	88,0	80,0	92,0
	0,0	12,0	60,0	88,0	100,0	91,7
	0,0	0,0	52,0	96,0	92,0	74,1
	0	16	44	72	92	80
	12	12	44	88,5	92	88
	0	0	32		84	100
	0		44		88	
	0		68			
Mean	3,3	6,6	48,3	88,6	90,4	87,4
SD	4,3	6,0	9,6	9,2	5,7	7,7

Table S14. Compilation of results obtained by students whose sample contained prilocaine: a) crude value b) values without outliers

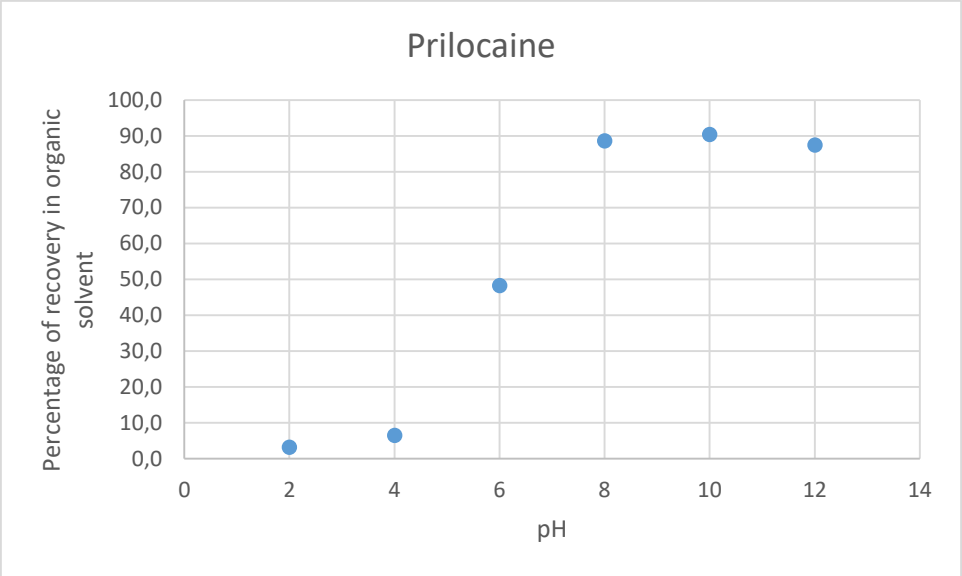


Figure S14. Extractability curve obtained by students whose sample contained prilocaine. (without outliers)

- Data obtained for quinidine sulfate:

a)

pH	2	4	6	8	10	12
	4	32	8	4	96	4
	4	96	23	96	88	92
	3,9	0,0	8,0	68,0	80,8	76,0
	0,0	3,9	1,2	77,8	70,4	92,3
	0,0	11,5	36,0	31,8	76,0	84,6
	0,0	12,0	20,0	87,5	96,0	72,0
	0,0	4,0	4,0	88,0	88,0	36,0
	8,0	0,0	0,0	60,0	96,0	80,0
	0,0	0,0	11,1	28,0	84,6	72,0
	16	4	16	68	76	80
	0	4	44	56	80	68
Mean	3,3	15,2	15,6	60,5	84,7	68,8
SD	5,0	28,3	14,2	28,7	8,9	26,3

b)

pH	2	4	6	8	10	12
	4	0	8	68	96	92
	4,0	3,9	23,0	77,8	88,0	76,0
	3,9	11,5	8,0		80,8	92,3
	0,0	12,0	20,0	87,5	76,0	84,6
	0,0	4,0	4,0	88,0	96,0	72,0
	0,0	0,0	11,1	60,0	88,0	80,0
	0,0	0,0	16,0	68,0	96,0	72,0
	8,0	4,0	44,0	56,0	84,6	80,0
	0	4			76	68
	16				80	
	0					
Mean	3,3	4,4	16,8	72,2	86,1	79,7
SD	5,0	4,6	12,8	12,7	8,0	8,7

Table S15. Compilation of results obtained by students whose sample contained quinidine sulfate: a) crude value b) values without outliers

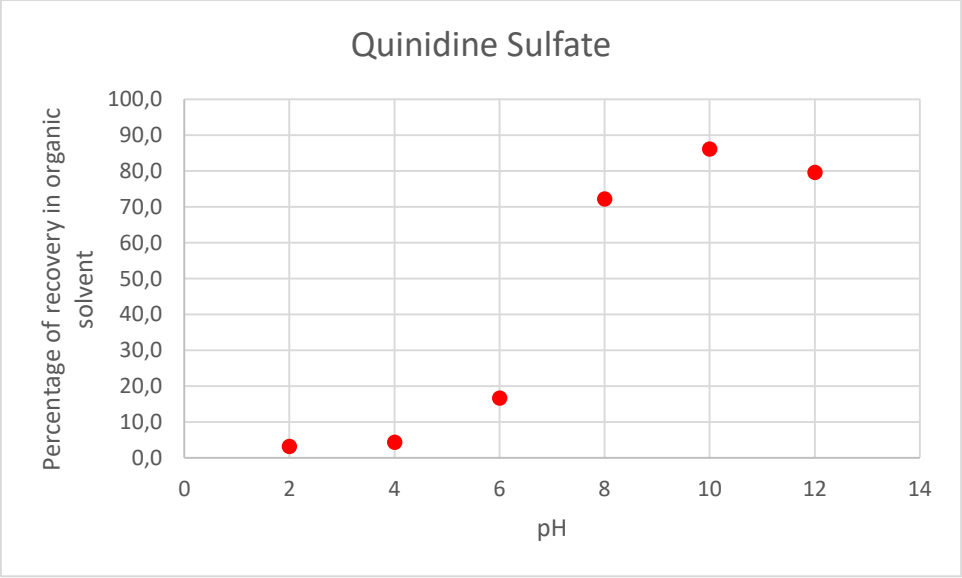


Figure S15. Extractability curve obtained by students whose sample contained quinidine sulfate. (without outliers)

9. LABORATORY REPORT

Each student receives one document for preparing their report (Figure S3). Once the experiments are done, the data are discussed, and the corresponding extractability profile is explained without mentioning the true identity of the tested compound only its physicochemical character such as a lipophilic acid or a salt of a lipophilic acid or a weak acid or a lipophilic amine and reported in the document.

Nom, Prénom :	Table : Groupe :	Date :

Travaux pratiques de Chimie Organique – Rapport pour la 6e séance

CODE DE L'ECHANTILLON (La lettre, le chiffre et la couleur)	

Solubilité dans l'eau :		Solubilité dans le solvant organique :	
pH de la solution :			

Tampon	1	2	3
pH			
Volume	mL	mL	mL
Couleur du tampon			
Pesée de l'échantillon	mg	mg	mg
Tare du ballon	g	g	g
Après extraction et évaporation			
Pesée du ballon	g	g	g
Masse de produit	mg	mg	mg
Pourcentage de produit extrait	%	%	%

Caractère acido-basique potentiel :	
Caractère Hydro/Lipophile potentiel :	
Justifier :	

Conclusion :

Le produit est

Indiquer vos valeurs sur le graphique commun.

Figure S16: Example of the original document to be filled by students throughout the laboratory session.

Name, Surname:	Table: Group:	Date:

Practical Session of Organic Chemistry – Report of the 6th session

Sample code (Letter, number and color)	

Solubility in Water:		Solubility in organic solvent:	
pH of the aqueous solution:			

Buffer	**1**	**2**	**3**
pH			
Volume	**mL**	**mL**	**mL**
Color of the buffer			
Sample weight	**mg**	**mg**	**mg**
Tare of the flask	**g**	**g**	**g**
After extraction et evaporation			
Evaporated flask weight	**g**	**g**	**g**
Weight of the residue	**mg**	**mg**	**mg**
Percentage of extraction	**%**	**%**	**%**

Acid/Base characteristics of the sample:	
Hydro/Lipophilic characteristics of the sample:	
Justification:	

Conclusion:

The sample is

Report your values on the common graph.

Figure S17: The English translation of the report is reported below.

10. ASSESSMENT

- Elements to be taken into account for evaluation of the report:

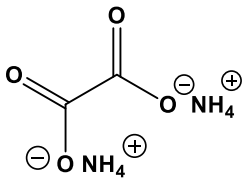
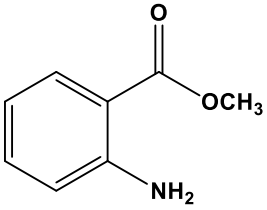
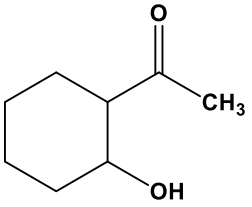
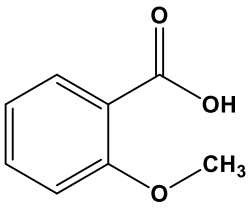
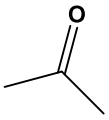
	Excellent	Minor improvement	Major improvement
Experimental			
Solubility in diethyl ether	Correct solubility	/	Wrong answer. Need more time for solubilization, or stir more the solution
Solubility in water	Correct solubility	/	Wrong answer. Need more time for solubilization, or stir more the solution
Percentage of extraction	Percentage is under 10% difference with the mean	Percentage is between 10% and 30 % difference with the mean. - Tare flask is incorrect -Separation of organic phase and aqueous phase need minor improvement -Need to use more $MgSO_4$ -Initial weight of the compound incorrectly weighted	Percentage is over 30 % difference with the mean. -Scale's tare incorrect - Tare flask is incorrect -Separation of organic phase and aqueous phase need major improvement -Need to use more $MgSO_4$ -Initial weight of the compound incorrectly weighted
Arithmetic			
Units' conversion	Correct answer	/	Wrong answer. Need to train more on the exercise seminars
Weight of residue	Correct answer	/	Wrong answer. Need to train more on the exercise seminars
Percentage determination	Correct answer	/	Wrong answer. Need to train more on the exercise seminars
Chemical Characteristics			
Acid/Base characteristics	Correct characteristics	/	Incorrect characteristics
Hydro/Lipophilic characteristics	Correct characteristics	/	Incorrect characteristics
Justification of these characteristics	Correct interpretation showing understanding of acid/base and	Good acid/base and hydro/lipophilic properties but with wrong interpretation	Confusion between an acid and a corresponding salt or a base and a corresponding salt

	hydro/lipophilic properties of the extracted compound		because extractability curves have similar shape
Conclusion	Correct conclusion	Not making the difference between strong and weak acid	Confusion between an acid and a corresponding salt or a base and a corresponding salt because the extractability is the same in relation to the pH

- Questions asked during evaluations:

Examination before a laboratory session

Question : For each compound complete the table (YES-NO). The three answers for each compound must be correct otherwise the grade is zero

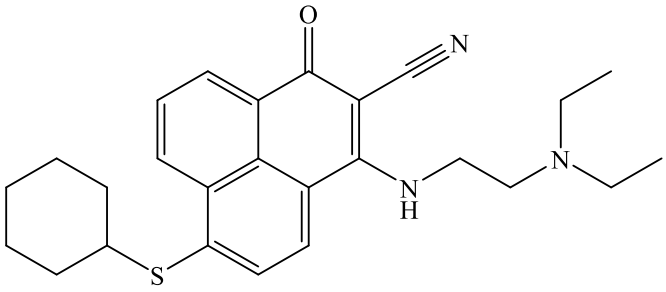
Compounds	Soluble in water	Extractable by an organic solvent and an aqueous acidic medium (HCl)	Soluble in an organic solvent
    			

-Final examination

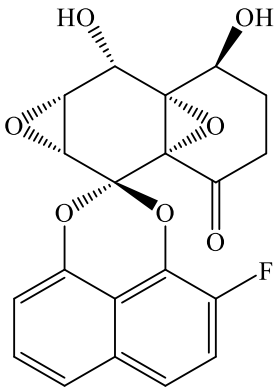
This first question regarding identification of groups is very important for the rest of the evaluation

Question: In each compound, indicate clearly and precisely the groups excepted the aromatic rings and the alkyl groups.

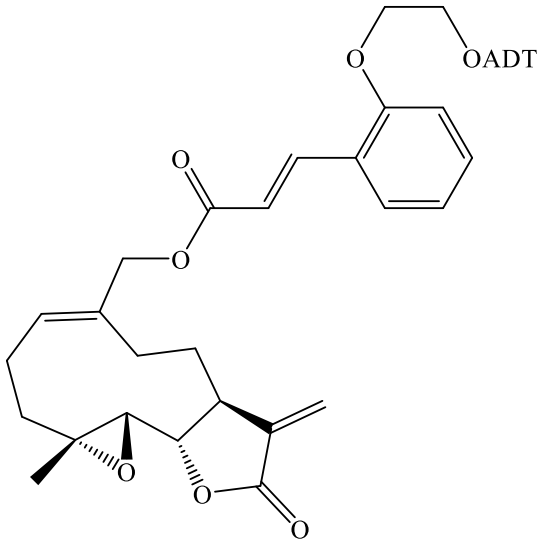
1



2

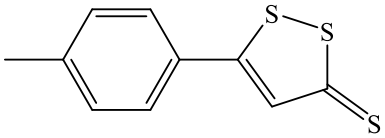


3



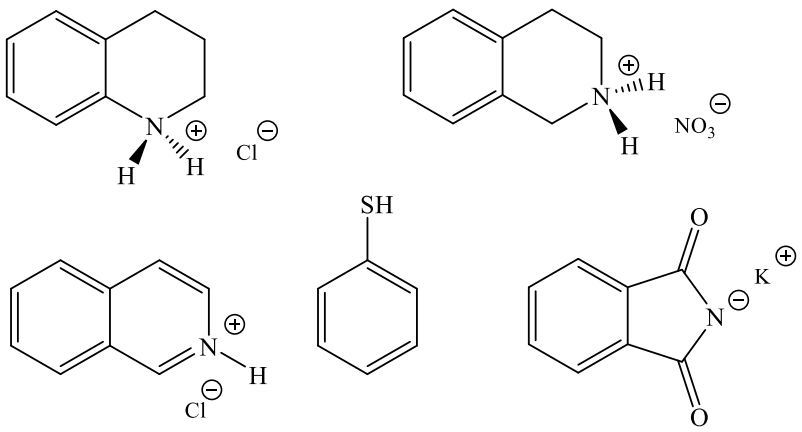
For information ADT =

do not identify

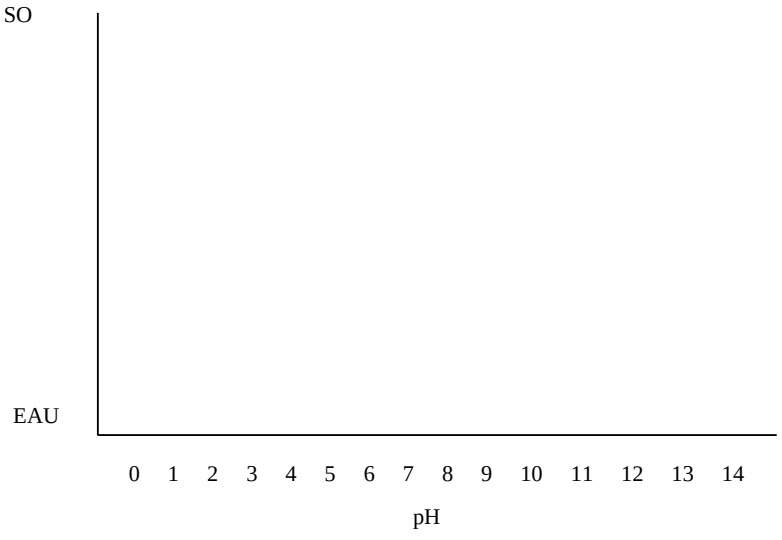


-Other question regarding extractability

Part A. Indicate precisely the group present in each compound and characterize the physicochemical properties (acid/base (conjugate or not), hydro/lipophilic) of the corresponding compound. Justify the answer.



Part B. On the graph, draw the corresponding curve of extractability for each compound (see above).



For the correction, if the group is not exactly identified in Part A, the rest of the question (Part B) is not taken into account