

# APPLICATION OF THE DYE ACID RED 88 FOR EXTRACTION-SPECTROPHOTOMETRIC ANALYSIS OF THE FENTANYL AND ITS DERIVATIVES

Romana JELÍNKOVÁ, Emil HALÁMEK, Zbyněk KOBLIHA  
romana.jelinkova@unob.cz

## Abstract

*In this work we observe the reactions of selected agents - fentanyl and its derivatives alfentanil, remifentanil and sufentanil - with a specific reagent resulting in the creation of ion pairs and we verify the possibility of reliable determination of those agents by the extraction spectrophotometry method in the sphere of visible radiation. The procedure is based on protonation of agents in the acid environment and their extraction in the form of an associate with the acid dye anion into an organic phase immiscible with water – chloroform. The ion associates of fentanyl and its derivatives with Acid Red 88 absorb the light at a maximum of  $\lambda_{max}$  518 nm.*

## Key words

*Fentanyl, UV/VIS spectrophotometry, extraction, azo-dye.*

## 1 Introduction

For several decades, the synthetic and opioid-like synthetic material, fentanyl, has been known to the general public and the general public. Its effects are mainly used in the field of medicine, but there are also known cases of its abuse among drug addicts. He also came to broader public awareness in 2002 when the Russian government decided to use this substance - precisely two of its derivatives - during the liberation of hostages in the Moscow theater, which was occupied by Chechen terrorists [1].

Fentanyl belongs to highly efficient agonistic opioids of anilidopiperidine type. The substance *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide was synthesised by Janssen, a Belgian chemist, for the first time in 1960 to be used as intravenous anaesthetic under the name Sublimaze. Several years afterwards, it was used on a worldwide scale. Due to its quick onset and relatively quick subsiding of its effect, it is used in neuroleptanalgesia at short-lasting surgeries [2].

Alfentanil, which is known under the commercial names Alfenta and Rapifen, is a highly efficient analgesic; it is the derivative of fentanyl with similar effects, quicker onset and a shorter period of effect [3,4]. It is used mainly as neuroleptanalgesic in outpatient surgery. In comparison with fentanyl, propitious pharmacokinetic properties are indicated [5]. Remifentanil is used as a quick and short-lasting analgesic administered as a complement in the form of remifentanil hydrochloride. The substance is used as short-lasting analgesic in outpatient surgery; on the other hand, it has no use in the cure of chronic pain [6,7].

Sufentanil white or almost white powder insoluble in water, but easily soluble in methanol and ethanol (96%) and it is known under the name Sufenta□ and Sufenta Forte [8]; furthermore, it is used in epidural and combined full anaesthesia. It has 10-times stronger analgesic effect than fentanyl and it is highly soluble in fats, which allows quick penetration into CNS. The duration of the effect is approximately 10 – 25 minutes. Concomitant undesirable side effects of these substances are nausea and chest wall rigidity [9]. The structure of interest analytes is shown (Fig. 1). At present, about 40 derivatives are known, for example:

acetylfentanyl, acrylfentanyl, cyclopentyl fentanyl,  $\alpha$ -methylfentanyl, *p*-fluorofentanyl, lofentanil, 3-methylthiofentanyl, ocfentanil, thiofentanyl and other [10].

The Dynex immunochemical test is used for orientative detection of fentanyl [12]. Mobile workstations of the integrated rescue system have hand held spectrometers available. Fentanyl and some of its derivatives are abused by drug addicts, and in the past several cases of abuse of these substances have been reported among athletes, so anti-doping control has to be done. For more accurate determination of this substance, stationary laboratories use, for example, liquid or gas chromatographs in conjunction with mass spectrometry [13].

The aim of the experiment was to find ideal conditions for the determination of fentanyl for the development of the methodology by extraction-spectrophotometric method. The studied analytes that contain in their molecules amino-group form ion-associates with suitable colouring agents, which are extracted with dissolving agents non-miscible with water (Fig. 2). A screening of appropriate azo dyes agents was performed which could be used in the chosen method. Over 70 reagents were tested. The dyes examined included, for example Acid Blue 113, Acid Blue 120, Acid Orange 51, Acid Red 114, Acid Yellow 14 and others, but exhibited deficiencies in the experiments in the form of foaming of the ionic partner, staining of the blank, low values of maximum absorption, etc.

An acidic nitrogen dye Acid Red 88 (CAS 1658-56-6) was used as a reactive agent for extractive spectrophotometric determination of analytes. It belongs to the group of azo-compounds, which have avid colouring due to  $\pi$ -delocalization of electrons in double bonds [14].

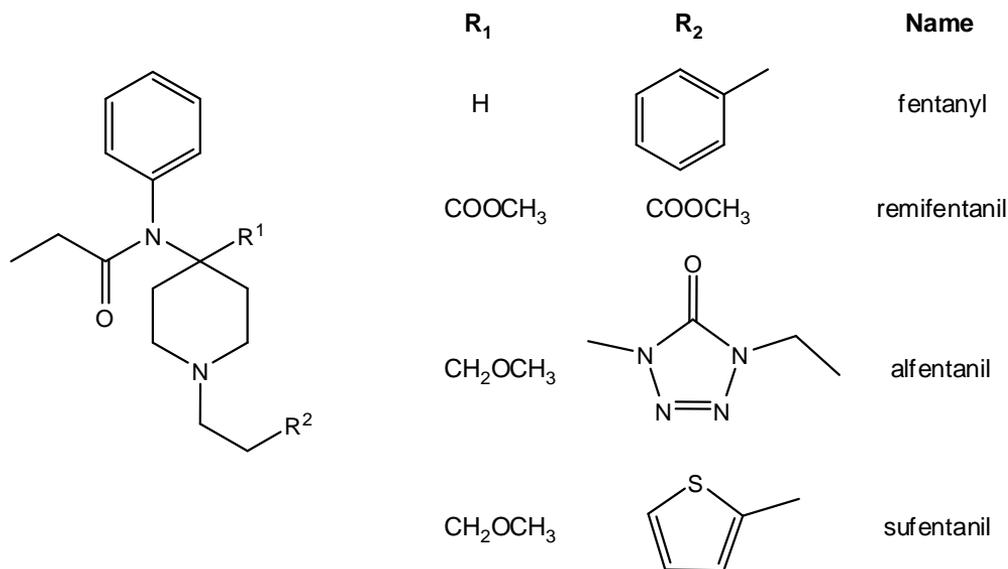


Figure 1

The structure of analytes: fentanyl, remifentanyl, alfentanyl and sufentanyl

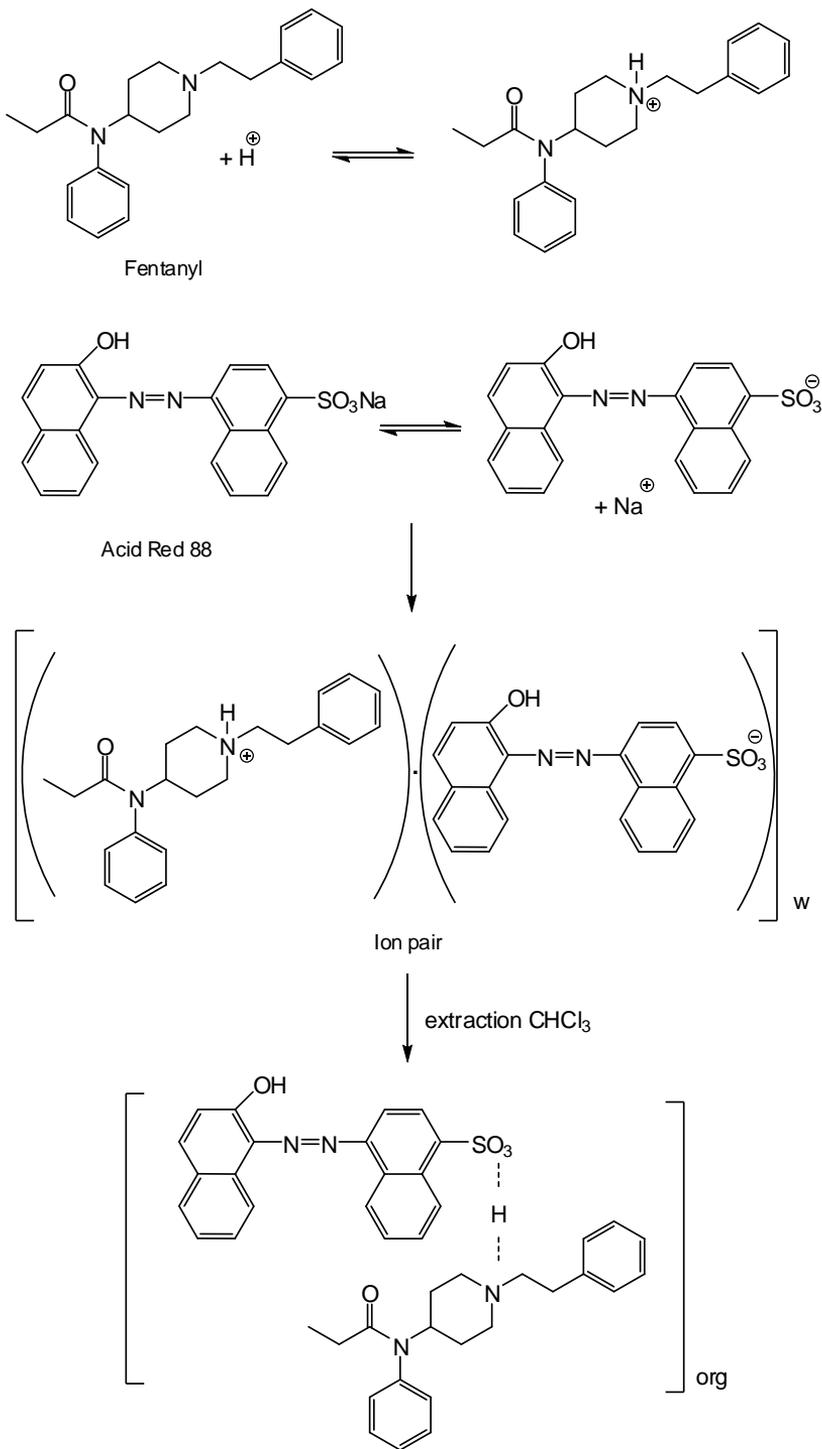


Figure 2

The scheme of the creation of the ion associates of fentanyl and the agent Acid Red 88 [11]

## 2 Experimental

### 2.1 Agents

#### 2.1.1 Substances used:

Fentanyl dihydrogenocitras, CAS 990-73-8, injection form, company Torrex Pharma Ltd, Prague, CZ, 1 mL of solution contains 50 µg of fentanyl.

Rapifen - Alfentanil hydrochloridum, CAS 69049-06-5, Janssen-Cilag Ltd, Prague, CZ, for intravenous administration 0.544 mg in 1 mL corresponds with 50 µg of alfentanil.

Remifentanil hydrochloridum, CAS 132539-07-2, medical injection form, Ultiva™, Glaxo Group Ltd Greenford, Middlesex, UK, 2 mg of remifentanil base in an ampoule.

Sufentanil dihydrogenocitras, CAS 60561-17-3, Torrex Pharma Ltd, Prague, CZ, for intravenous administration; 75 µg sufentanil dihydrogenocitras corresponds with 50 µg sufentanil in 1 mL.

To conduct extensive screening of dyes, the agent Acid Red 88, CAS 1658-56-6, (C<sub>20</sub>H<sub>13</sub>N<sub>2</sub>NaO<sub>4</sub>S), M.W. 400.38 was selected to determine selected opiates.

The remaining chemicals were used in the purity p.a. from the company Sigma-Aldrich.

Other chemicals used: HCl (Hydrochloric acid), KH<sub>2</sub>PO<sub>4</sub> (Potassium dihydrogenphosphate), Na<sub>2</sub>HPO<sub>4</sub> (Disodium hydrogenphosphate), C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> (Citric acid), NaOH (Sodium hydroxide), their stock solutions according to Merck manual were prepared.

#### 2.1.2 Instruments:

The double beam UV/VIS spectrometer Unicam – Helios α. Unicam Instruments, Cambridge, UK, was used together with the glass cuvette, type 6030, optical length 10 mm (HELLMA GmbH & Co, Germany).

The check of buffer solutions was conducted on the pH meter Hanna 213 with the electrode type HI 1131 B (Hanna Instruments GmbH, Germany).

Concerning the low concentration of the used injection solutions, the samples were lyophilised using the lyophilisator Hetosicc Freeze Dryer FD 3, Heto-Holten A/S, Gydevang 17-19, DK – 3450 Allerød, Denmark.

### 2.2 Procedure

The absorbance curve of ion associates was measured using the functional dependency of the absorbance values on the wavelength in the region of visible spectrum.

2.8 mL of buffer solution with the pH ranging from 1.1 to 6 (Table I.) was pipetted into a test tube, then 0.1 mL of the water solution of a dye and 0.1 mL of analytes at the concentration of 1.10<sup>-3</sup> mol.L<sup>-1</sup> were added. After adding 3 mL of chloroform, the solution was extracted by shaking for the period of 2 minutes [15]. Subsequently, the water layer was suctioned and the values of ion associates in the organic phase against pure chloroform were measured. Out of the measured spectrum, the highest value of absorbance marked as λ<sub>max</sub> was read [16,17]. The measured values of maximum absorbance created by ion associates for Acid Red 88 were 518 nm.

To ascertain the dependence of the absorbance on the pH values of buffer solutions, 2.8 mL of buffer solution with the pH ranging from 1.1 to 6 was pipetted at 0.5 units. Subsequently, the absorbance of the chloroform extract of ion associate at the wavelength λ<sub>max</sub> was measured. Out of the obtained results, the buffer solution of the pH at which usage the highest values of the absorbance of the sample and the lowest absorbance of a blank experiment

were measured was selected. The experiment was conducted with the solution of analytes at the concentration of  $1 \cdot 10^{-3} \text{ mol.L}^{-1}$ .

*Table I*  
*The preparation of 100 mL buffer solution*

pH	Citrate solution (mL)	0,1-M HCl (mL)
1,1	4,8	95,2
1,5	22,2	77,8
2	30,6	69,4
2,5	35,4	64,6
3	40,3	59,7
3,5	46,8	53,2
4	56	44
4,5	71,9	28,1

pH	Na <sub>2</sub> HPO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>
5	0,95	99,5
5,5	3,9	96,1
6	12,1	87,9

To ascertain the optimal concentration of a dye, the water solution of the dye with the concentration ranging from  $1 \cdot 10^{-4}$  to  $1 \cdot 10^{-3} \text{ mol.L}^{-1}$  with a unit of  $0.1 \cdot 10^{-3} \text{ mol.L}^{-1}$  was pipetted into a test tube and refilled to reach the volume of 3 mL. Subsequently, the chloroform phase at  $\lambda_{\text{max}}$  for each specific dye was measured. After adding values into the graph, the concentration of the dye indicating the highest value of absorbance was selected.

The calibration curve is determined by measuring the dependence of absorbance on the concentration of analytes. 2.8 mL of the buffer solution of the optimal value for each separate agent, 0.1 mL of a dye and 0.01 – 0.1 mL of the solution of the analytes at the concentration ranging from  $1 \cdot 10^{-4}$  to  $1 \cdot 10^{-3} \text{ mol.L}^{-1}$  were pipetted into a test tube, then 3 mL of chloroform was added and the extraction was being conducted for the period of 2 minutes. Following the suction of the water phase, the absorbance of the ion associate in chloroform at the optimal pH was measured.

The composition of the ion associate in the organic phase was measured from the functional dependence  $A = f(x/L)$ , where  $x/L$  stands for the molar fraction of a dye. 2.8 mL of the buffer solution at an optimal pH was pipetted into a test tube; subsequently, 20 - 180  $\mu\text{L}$  of a dye with the unit of 20  $\mu\text{L}$  was pipetted and filled with the amount of the water solution of analytes to reach the volume of 200  $\mu\text{L}$ . After the extraction and the suction of a water layer, the absorbance of the ion associate in chloroform was measured and the proportion ratio [18,19] was read in the place of local extreme.

### 3 Results and Discussion

It is evident from the evaluation of studied dependencies that the ratio of the selectivity to ascertain the measured substances corresponds with the different distribution of ion associates between water and organic phases. Using the method, optimal experimental

conditions influencing the course of reactions were studied - the wavelength of the absorption maximum of the ion pairs in the  $\lambda_{\max}$  extract, the pH of the solutions used (Fig. 3), and the extraction time are observed at normal laboratory temperature.

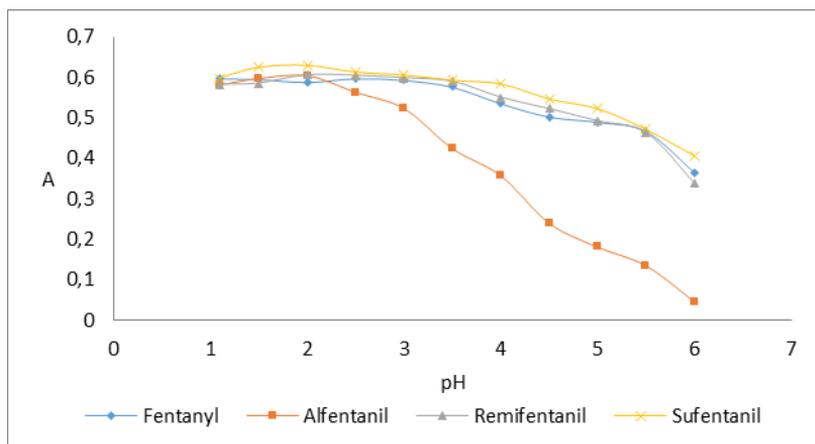


Figure 3

*Dependence of the absorbance of the extracts of ionic associates on the pH of the environment*

Dependence of the absorbance on the concentration of the selected dye was determined, the calibration line (Fig. 4) and absorption dependence on the molar fraction were determined.

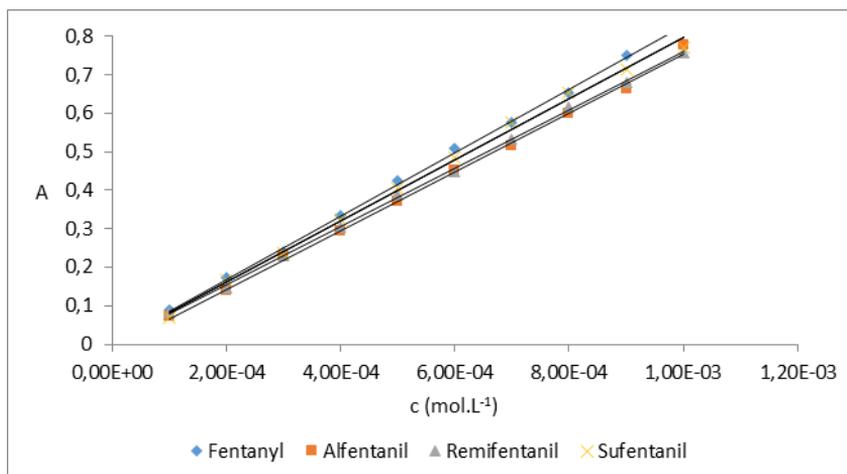


Figure 4

*The calibration lines of the ionic associates with Acid Red 88 reagent  
Concentration range of the basic analyte solution used  $1.10^{-4} - 1.10^{-3} \text{ mol.L}^{-1}$*

The predicted stoichiometric ratios of the studied compounds were also verified. Under the appropriate reaction conditions, detection limits and assay limits were calculated for the fentanyl ion associates with the reagents tested. The sensitivity of the analytical method is

declared by the directive of the calibration dependence of two variables. The extraction spectrophotometry carried out in the measured concentration range of the test substances complies with the Lambert-Beer Law. The method chosen has not demonstrated the possibility of selective determination of individual fentanyl derivatives.

The separate values are stated in the Table II.

Table II

*The parameters of ion associates and extractive spectrophotometric determination of fentanyl, alfentanil, remifentanil and sufentanil with the dye Acid Red 88*

Parameter	Fentanyl	Alfentanil	Remifentanil	Sufentanil
$\lambda_{\max}$ [nm]	518	518	518	518
pH <sub>opt</sub>	2.5	2	2	2
X/L	1	1	1	1
R <sup>2</sup>	0.9992	0.9981	0.9993	0.9969
S <sub>q</sub>	0.0026	0.0029	0.0025	0.0021
q	0.0049	-0.0094	0.0029	0.0048
LOD [mol.L <sup>-1</sup> ]	1.09.10 <sup>-5</sup>	1.17.10 <sup>-5</sup>	1.19.10 <sup>-5</sup>	1.13.10 <sup>-5</sup>
LOQ [mol.L <sup>-1</sup> ]	3.64.10 <sup>-5</sup>	3.92.10 <sup>-5</sup>	3.96.10 <sup>-5</sup>	3.78.10 <sup>-5</sup>

$\lambda_{\max}$  - maximum absorbance value, pH – optimal potential of hydrogen, X/L - molar fraction of the reagent, R<sup>2</sup> - Reliability value, S<sub>q</sub> - standard deviation of the coefficient q, q - coefficient of displacement of the starting point on the y axis, LOD – detection limit, LOQ - limit of determination

#### 4 Conclusion

The method was developed to ascertain the selected groups of the substances of the opioid type. The optimal conditions of the creation of the colourfully distinctive ion associates of fentanyl, alfentanil, remifentanil and sufentanil with the selected azo dyes Acid Red 88 were ascertained and the homogeneous procedure of *the extractive spectrophotometric determination of selected analytes* was developed. Analytes can be ascertained even at very low concentration. The chosen and verified method is applicable for quick, accurate and financially undemanding determination of given opioids.

#### Résumé

*Even in the era of modern instrumentation, it is often necessary to choose a relatively inexpensive, simple and rapid method of determination of interest, which can be used in the described method of extraction-spectrophotometric determination of the opioid character group. The method was created for use in the Deployable Laboratory of the Czech Army primarily.*

#### References

- [1] RICHES, R. J. et. al. *Journal of Analytical Toxicology*. 2012, 36(9), 647-656. ISSN 0146-4760.
- [2] STANLEY, H. T., D. T. EGAN and H. van AKEN. *Anest. Analg.* 2008, 106, 2. ISSN 0099-8125.

- [3] LÜLLMANN, H., K. MOHR and M. WEHLING. *Farmakologie a toxikologie*. 2.<sup>ed</sup> Praha: Grada Publishing, 2004. ISBN 80-247-0836-1.
- [4] MATHEW, J. and K. KENDALL. U. S. Patent 7,208,604 B2., 2007.
- [5] LEE, G.M. and J. KOREAN. *Anaesthesiol.* 2011, 61(4), 202. <https://doi.org/10.4097/kjae.2011.61.4.292> (assessed: May 19, 2014).
- [6] BILLARD, V. and F. S. SERVIN. Remifentanil and Other Opioids. *Handbook of Experimental Pharmacology*, 2008, 182, 283.
- [7] HESS, L. *Remedia*. 1992, 263, 2(4-5).
- [8] Český lékopis. Praha: MZ ČR, Grada, 2002.
- [9] KIZILKAYA, M. et al. *Anaesth. Analg.* 2003. Available at: <http://www.anesthesiaanalgesia.org/content/98/4/1062.full?related-urls=yes&legid=anesthanalg;98/4/1062> (assessed: February 11, 2011)
- [10] EMCDDA. 2017. Available at: [http://www.emcdda.europa.eu/data/stats2018\\_en](http://www.emcdda.europa.eu/data/stats2018_en) (assessed: April 21, 2018)
- [11] JELÍNKOVÁ, R. *Extrakční spektrofotometrie fentanylu a jeho derivátů*. Brno, 2014. Disertační práce. Univerzita obrany Brno, ÚOPZHN.
- [12] Testy na drogy. Novinka: test na fentanyl. *Drugs & Forensics Bulletin Národní protidrogové centrály*. 2011. 4. ISSN 1211-8834.
- [13] ADAMOWICZ, P. and A. SKULSKA. Determination of fentanyl in hair and urine of a person using transdermal patches. *Problems of Forensic Sciences*. 2013, vol. 94, 578–589. ISSN 1230-7483.
- [14] KOBLIHA, Z., E. HALÁMEK a J. SOUČEK. Skupinový test k určení aminických sloučenin. Patent ČSFR; 277616. 1992.
- [15] JELÍNKOVÁ, R., Z. KOBLIHA a E. HALÁMEK. *Chemické listy*. 2015, 109(2), s. 135–139. ISSN 0009-2770.
- [16] JANDERA, P. *Atomová a molekulová spektroskopie se zaměřením na stopovou analýzu kontaminantů*. 3.<sup>ed</sup> Pardubice: Univerzita Pardubice, 2011. ISBN 978-80-7395-392-8.
- [17] ČŮTA, F. *Instrumentální analýza*. 1.<sup>ed</sup> Praha: SNTL, 1986.
- [18] HALÁMEK, Emil a Zbyněk KOBLIHA. *Collection of Czechoslovak Chemical Communication*. 1993, 58(2), 315-319. ISSN 0010-0765.
- [19] LAŠKOVÁ, M. a M. BLEŠOVÁ. *Čes. Slov. Farm.* 2003. 252, 52(5). ISSN 1210-7816.