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REVIEW ARTICLE

THE POSSIBILITY OF IDENTIFYING SELECTED OPIOIDS BY SPECTRAL ANALYSIS

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Summary

The ability of the examined compounds to create ion pairs with the selected anionic dyes, allows their determination by the method of extraction spectrophotometry in the visible radiation. These ion pairs are extractable with an organic solvent immiscible with water. The procedure is based on protonisation of the studied compounds in acidic environment, and consequently their extraction in the form of associate with the anion of the acid dye into the organic phase, in this case in chloroform.

Mentioned methods of analysis may be advantageously used for simple and express determination of fentanyl, alfentanil and remifentanil.

Key words: Fentanyl; alfentanil; remifentanil; ion pairs; UV/VIS spectrophotometry; azo dyes.

INTRODUCTION

Chechen separatists occupied Dubrovka theater in Moscow in October 2002. Russian Special Forces employed narcotic gas to incapacitate attackers during the rescue operation [1]. This substance comes from Kolokol 1 programme and has been previously used by KGB specialists as psychoactive gas. The effects of respiratory suppression and suffocation had killed over hundred people. Despite all the doubts of experts and with support by the later expression of the Russian Ministry of Health it most likely was Fentanyl which has not yet been reported on the human organism with lethal consequences. Dehydration, starvation and psychological stress also participated on the critical condition of hostages in enclosed space of the theater. Unauthorized sources indicate that there were probably used derivatives of 3-methylfentanyl, carfentanil or alfentanil [2, 3]. Some hypotheses admit the use of remifentanil [4]. There is a need for rapid identification in the case of abuse of these substances by terrorist groups. Spectral techniques in the UV/VIS can be used in such circumstances. Determination of these opioids in biological materials is currently carried out mainly by using techniques of high performance liquid chromatography or gas chromatography coupled with mass spectrometry, especially in the medical field [5, 6]. Neither the Army of the Czech Republic nor the Allied Forces have enough sophisticated means for a timely quantitative analysis of Fentanyl and its derivatives. There is a need to find new methods which are fast, reliable, and easy to use in terms of military field analysis due to the time and economy demands of some modern chromatographic and spectroscopic methods. Requirements of quantitative determination of the opioid nature substances meet

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spectral analysis in the UV/VIS region, which has been successfully used, for example, in determining some chemical warfare agents. [7, 8].

Fentanyl is primarily used in medical and veterinary practice as an anesthetic, but may be misused for its incapacitating properties as a potential weapon. In recent years, fentanyl has been abused among drug addicts as a substance easily interchangeable with heroin or mixed with some other chemicals. Danger of confusion of fentanyl with heroin lies mainly in the fact that it is about 50 times more potent than heroin and it is able to cause significant rigidity of chest muscle. In addition to respiratory depression, the use of fentanyl usually results in low blood pressure [9, 10].

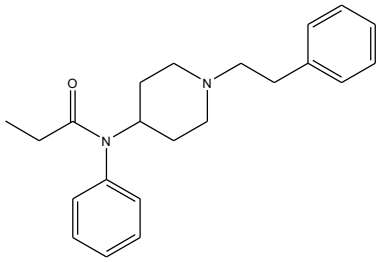

Figure 1. *N*-(1-(2-phenylethyl)-4-piperidinyl)-*N*-phenylpropanamide structure formula – Fentanyl

Fentanyl is a very effective agonists acting synthetic opioid, among which belong pethidine, levometadon, piritramide. It is usually administered intravenously. The analgesic effect usually emerges in one minute after the injection and the duration of the effects is approximately 30 minutes [9]. Analgetic effect lasts up to two hours. Short duration of analgesia following intravenous administration of fentanyl classifies the substances suitable for analgesia prior to surgery, as well as for pain relief in acute myocardial infarction. A disadvantage of the use of fentanyl in medical practice is a very small difference between a safe and an effective lethal dose [11]. Respiratory depression may last longer than the analgesic effect and may reappear in the postoperative period, which requires continuous monitoring of the patient.

The existence of other derivatives of fentanyl, such as alfentanil, remifentanil, sufentanil, lofentanil, ohmefentanyl, carfentanil, 3-methylfentanyl, thiofentanil and several others, is known.

In the pharmaceutical industry, Alfentanil is known as Alfenta® or Rapifen. This derivative exhibits about 10 times less efficiency compared to fentanyl, but shows 4x faster onset (about 60 seconds) and a faster offset of action. It accumulates less in tissues compared to fentanyl and it has favorable pharmacokinetic properties [9, 10, 11]. It was synthesized in laboratories Janssen Pharmaceutica in 1976. It causes fewer cardiovascular complications than fentanyl and remifentanil, but it can cause respiratory problems, therefore it is necessary to ensure the control of vital signs during its usage. It is usually administered parenterally to patients. It belongs to prohibited drugs according to List II in the USA, however, these substance,s despite its considerable effects on the human body, are not included in the list of the Convention on the Prohibition of Chemical Weapons.

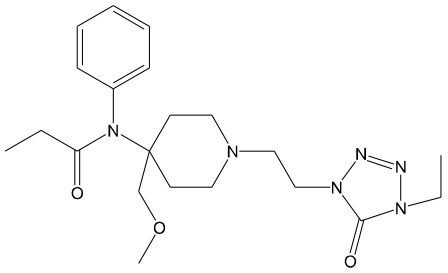

Figure 2. *N*-{1-[2-(4-ethyl-5-oxo-4,5-dihydro-1*H*-1,2,3,4-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl}-*N*-phenylpropanamide structure formula - Alfentanil

Remifentanil contains an ester bond in its molecule and is cleaved by non-specific esterases in plasma and tissues. It is administered as the hydrochloride salt of remifentanil to adult patients as an intravenous infusion at doses ranging from 0.1 to 0.5 (μg/kg)/min [9]. In blood it is relatively rapidly inactivated by esterases into inactive metabolites. Hence, it has very short duration of action and good controllability of exposure. The substance is used for short-term analgesia in ambulatory procedures, with almost zero use in the treatment of chronic pain. The side effects of remifentanil include dependence, bradycardia, respiratory problems and nausea [14, 15]. It can be administered only under conditions of fully secured monitoring and support of respiratory and cardiovascular function.

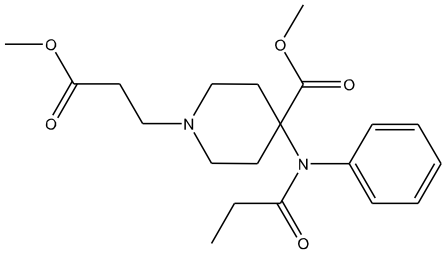

Figure 3. Methyl 1-(3-methoxy-3-oxopropyl)-4-(*N*-phenylpropanamido)piperidine-4-carboxylate structure formula - Remifentanil

THE CHEMICALS AND EQUIPMENT

- Fentanyl dihydrogen citrate, CAS 990-73-8, formulated injection drug by Torrex Pharma Ltd, Prague, CZ, 50 μg/ml contains 0.0785 mg of dihydrogen citric acid salt.
- Rapifen – alfentanil hydrochloride, CAS 70879-28-6, Janssen-Cilag Ltd, Prague, CZ, for intravenous administration, 0.544 mg in 1 ml corresponds to 0.50 mg of alfentanil in 1 ml.
- Remifentanil hydrochloride, CAS 132539-07-2, formulated injection drug under the trade name Ultiva ™, Glaxo Group Ltd, Greenford, Middlesex, UK, 2 mg in one vial.
- Acid Orange 8, CAS 5850-86-2, ($C_{17}H_{13}N_2NaO_4S$), M. W. = 364.35
- Acid Red 88, CAS 1658-56-6, ($HOC_{10}H_6N{=}NC_{10}H_6SO_3Na$), M. W. = 400.39
- Crocein Orange G, CAS 1934-20-9, ($C_{16}H_{11}N_2NaO_4S$), M. W. = 350.324
- Ethyl Orange, natrium salt, CAS 633-96-5, ($C_{16}H_{11}N_2NaO_4S$), M. W. = 355.39
- Potassium dihydrogen phosphate, CAS: 7778-77-0, Merck p.a.
- Disodium hydrogen phosphate dihydrate CAS 7558-79-4, Merck, p.a.
- Citric acid, CAS 5949-29-1, Lach-ner, p.a.
- NaOH, CAS 1310-73-2, Lach-ner, p.a.
- HCl, CAS 7647-01-0, Penta, p.a., 36%
- Chloroform (stabilized), CAS 67-66-3, Merck, p.a.

DEVICES USED FOR WORK

- Double-beam spectrophotometer Spekol α-Helios – Unicam (Unicam Instruments, Mercers Row, Cambridge CB5 8HY, UK with Vision 32 programme - Unicam Instruments.
- pH meter Hanna 213 (Hanna Instruments GmbH, Germany).
- glass cell, type 6030, path length of 10 mm (HELLMA GmbH & Co, Germany); micropipettes (Eppendorf AG, 22331 Hamburk, Germany).
- Lyophilizer Hetosicc Freeze Dryer FD 3 (Heto-Holten A/S, Gydevang 17-19, DK – 3450 Alleröd, Denmark).

WORKING PROCEDURES

Reagents suitable for determining the substance of interest - fentanyl and its derivatives - are nitrogen dyes, which are assigned to the group of azo compounds. Presence of electron acceptor substituents withdrawing electron out of the aryl core and amplifying the positive charge on the outermost nitrogen atom of the azo group allows entry into the reaction in the form of electrophilic reagents. Azo dyes are solid and chemically relatively stable substances. After performing extensive screening of 395 dyes, some responsive, structurally similar agents were selected which, together with fentanyl and its derivatives, produce significantly colored ion associates

measurable in standard absorbance values [16]. Chosen dyes: Acid Orange 8, Acid Red 88, Crocein Orange G, Ethyl Orange, sodium salt.

Absorbance curve was determined by measuring the ion associates functional dependence A = f (λ) after preparation of samples by lyophilization and extraction time optimization. There is 2.8 ml of citrate buffer pipetted in the test tube (the pH range 1 - 6) and 100 ml of reagents is added together with the same amount of aqueous solution with a concentration of the examined substances of 1.10^{-3} mol/l. After adding 3 ml of chloroform, substances are extracted for 2 minutes. This is then followed by suction of the aqueous layer and the chloroform layer measurements against pure chloroform. The measuring range is determined by the extent of the visible spectrum, i.e. 400-700 nm. Another part of the measurement is then performed with a value showing the highest absorbance of ion associate within that range. The absorbance is then marked as λ_{max} [17, 18].

The effect of pH on the formation of ion associates bases with dyes was studied by measuring the dependence A = f (pH). 2.8 ml of citrate buffer was pipetted with an interval of 0.5 pH units in the range of 1.5 - 6, and then the absorbance of the chloroform extract of ion associates was measured at the detected wavelength λ_{max}. An optimal buffer was then collected with a pH at which the measured absorbance of the sample had the highest value (Tab. 1).

Optimum dye concentration for the formation of ion associates in the aqueous phase was determined by measuring the dependence A = f (c). 10-100 ml of an aqueous dye solution with a concentration of 1.10^{-3} mol/l was pipetted in the test tube with an interval of 10 ml and water was added to the volume of 100 ml. Subsequently a chloroform phase at λ_{max} was measured for the selected dye. For further measurement we chose a dye concentration, which the highest absorbance value corresponds to.

The next step was to construct the calibration curve that is defined as a dependence A = f(c). 2.8 ml of buffer, 100 µl of aqueous dye solution and 10-100 µl of analyte solution was pipetted in the test tube with an interval of 10 µl. After that 3 ml of chloroform were added, extracted by shaking for the duration of 2 minutes. Upon suction of aqueous phase, the absorbance of the ion associate in chloroform at λ_{max} was measured.

Table 1. Parameters of ion associates and extraction spectrophotometric determination of fentanyl (I), alfentanil (II) a remifentanil (III) with reagents Acid Orange 8 (AO 8), Acid Red 88 (AR 88), Crocein Orange G (COG) a Ethyl Orange, sodium salt (EO).

Analyte	Reagent	λ_{max} [nm]	pH_{opt}	X/L	R^2	q	Є	LOD [mol/l]	LOQ [mol/l]
I	AO 8	495	1.5	1	0.9964	0.0095	19 920	$1.13.10^{-5}$	$3.78.10^{-5}$
I	AR 88	518	2.5	1	0.9992	0.0049	17 940	$1.09.10^{-5}$	$3,64.10^{-5}$
I	COG	486	2.5	1	0.9978	-0.0021	12 990	$1.7.10^{-5}$	$5.69.10^{-5}$
I	EO	435	3.5	1	0.997	-0.03	19 800	$1.33.10^{-5}$	$4.43.10^{-5}$
II	AO 8	494	2	1	0.9988	-0.0096	17 490	$1.2.10^{-5}$	$4.0.10^{-5}$
II	AR 88	518	2	1	0.9981	-0.0094	18 150	$1.17.10^{-5}$	$3.92.10^{-5}$
II	COG	485	1.1	1	0.9967	-0.0012	12 180	$1.68.10^{-5}$	$5.61.10^{-5}$
II	EO	435	3	1	0.9937	0.0061	10 860	$5.16.10^{-6}$	$1.72.10^{-5}$
III	AO 8	494	3	1	0.9983	-0.0121	18 420	$1.18.10^{-5}$	$3.93.10^{-5}$
III	AR 88	518	2	1	0.9993	0.0029	18 180	$1.19.10^{-5}$	$3.96.10^{-5}$
III	COG	485	2.5	1	0.9981	-0.0055	12 270	$1.73.10^{-5}$	$5.77.10^{-5}$
III	EO	435	3.5	1	0.9982	0.001	9 540	$2.00.10^{-5}$	$6.67.10^{-5}$

Parameters: pH_{opt} = optimal pH for extraction of ion assasiates, X/L = the molar ratio of analyte and dyes, R^2 = correlation coefficient, q = offset calibration curve on the axis y, Є = molar absorption coefficient calculated from the dependence A=f(c), LOD = limit of detection, LOQ = limit of quantification [19].

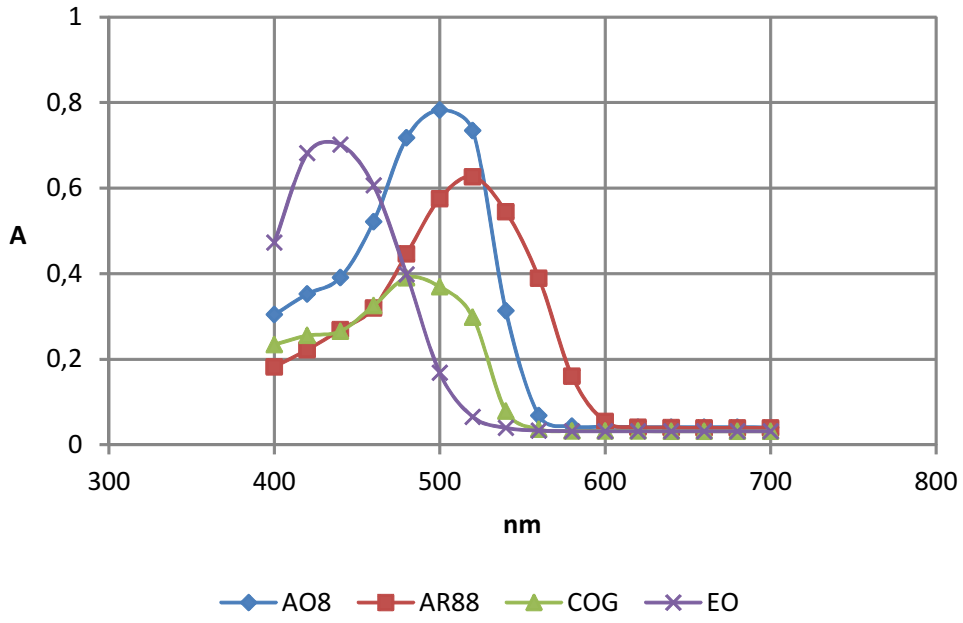

Figure 4. Fentanyl - dependence of absorbance of extracts of ion associates of fentanyl on the wavelenght

Stoichiometry of ion associate in the organic phase was then determined by continuous variation from the functional dependence A = f (xL), where xL is the molar fraction of counter ions. 2.8 ml of buffer solution with optimum pH was pipetted, then 20 to 200 ml of dye was added at the interval of 20 ml and adequate amounts of aqueous solutions at the selected concentration were filled up to the volume of 200 ml. After that 3 ml of chloroform were added, extracted by shaking for the duration of 2 minutes. Upon suction of aqueous phase the absorbance of the ion associate in chloroform at λ_{max} was measured.

All measurements were performed 3 times.

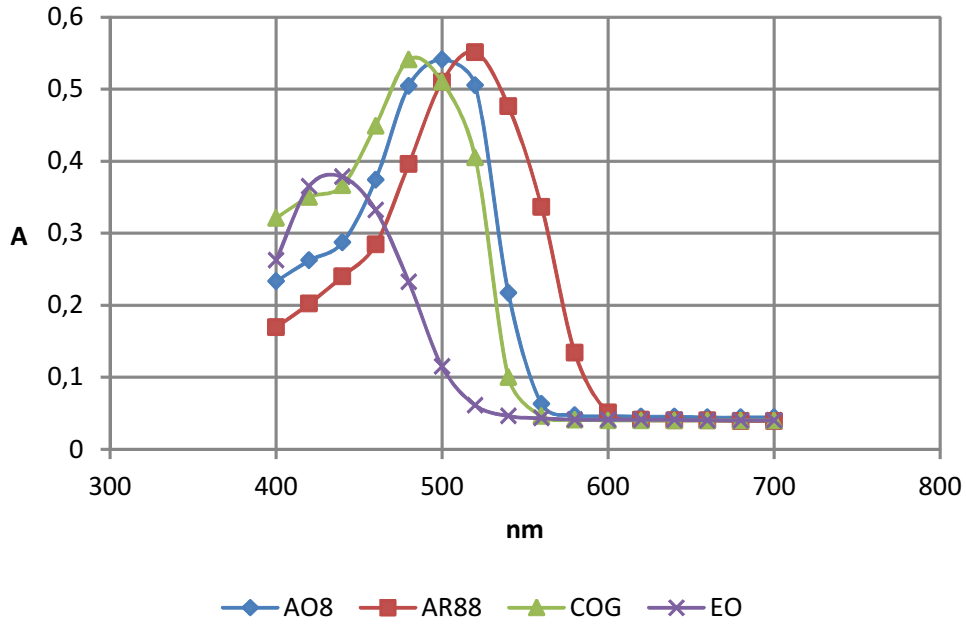

Figure 5. Alfentanil - dependence of absorbance of extracts of ion associates of alfentanil on the wavelenght

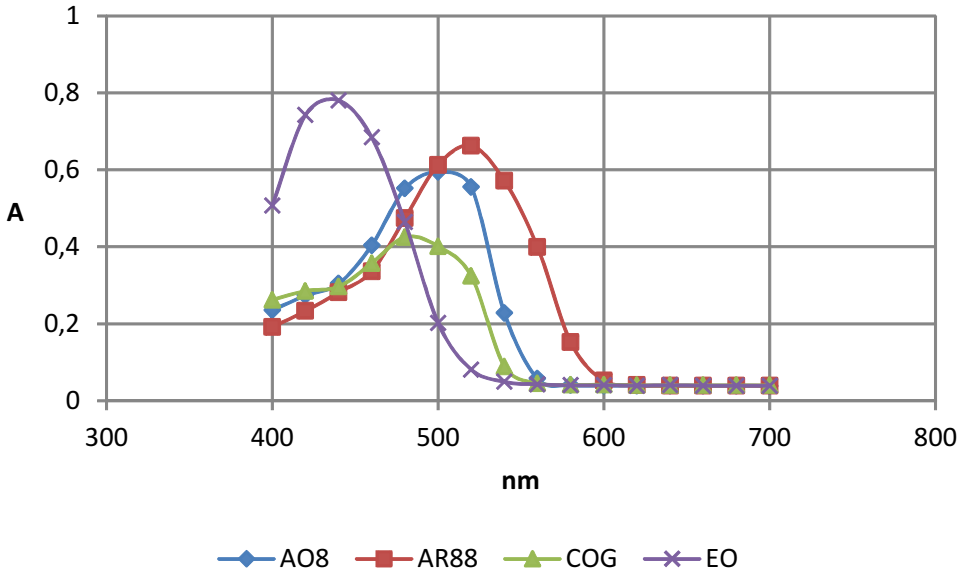

Figure 6. Remifentanil - dependence of absorbance of extracts of ion associates of remifentanil on wavelenght.

RESULTS AND DISCUSSION

The ion associates derived from the reaction between fentanyl and its derivatives and chosen dyes, absorbance of light at a wave length listed in Tab 1.

The buffer solution for particular analytes and reagents that showed the highest absorbance value (λ_{max}) was chosen based on monitoring the optimal dependence of solution absorbance on pH Test compounds and showed higher absorbance values especially in the acidic environment, the pH range 1.1 to 3.5 (see Tab. 1). In order to determine unknown concentrations of the samples the calibration straight lines have been plotted in the concentration range $c = 1.10^{-4} - 1.10^{-3}$ mol/l. The identified absorbances correlate with the values of concentrations of individual agents.

The method of continuous variation serves for detecting dependance of absorbance on molar fraction, i.e. the optimal ratio of individual components. Fentanyl, alfentanil and remifentanil provide ion associates with all reagents identically in the ratio 1: 1.

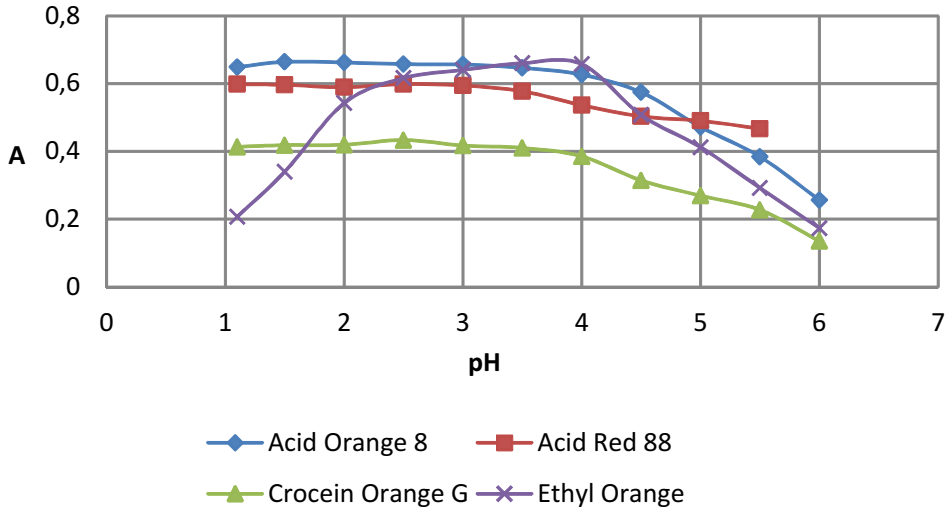

Figure 7. Fentanyl - dependence of absorbance of extracts of ion associates of fentanyl on the pH.

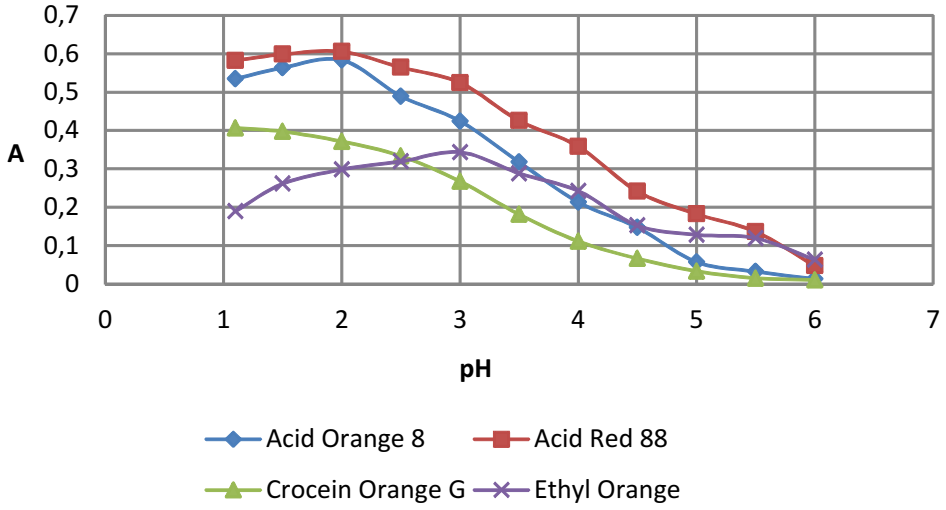

Figure 8. Alfentanil - dependence of absorbance of extracts of ion associates of alfentanil on the pH.

Substances of interest react with various reagents differently, so for their particular reliable determination it is possible to select a reagent whose parameters have clearly the lowest limits of detection and quantification as the graph and table show. The carried out experiments show that a suitable reagent for determining fentanyl and alfentanil by spectral UV/VIS analysis is the dye Acid Red 88. Dyes Acid Orange 8 and Acid Red 88 exhibit, with respect to the limits of detection and quantification, approximately equal values for determining remifentanil. It is thus possible to use both of them with an advantage.

CONCLUSION

The experience shows that fentanyl is included among incapacitating substances and its derivatives are agents which can be misused by drug addicts, during armed conflicts or terrorist attacks. The need to find simple and innovative methods focused on their prompt and reliable indicative determination is obvious. The UV/VIS spectrophotometry does not make high demands on an operating environment. The achieved results of the experiment have proved that the selected method enables a prompt and accurate identification of examined agents.

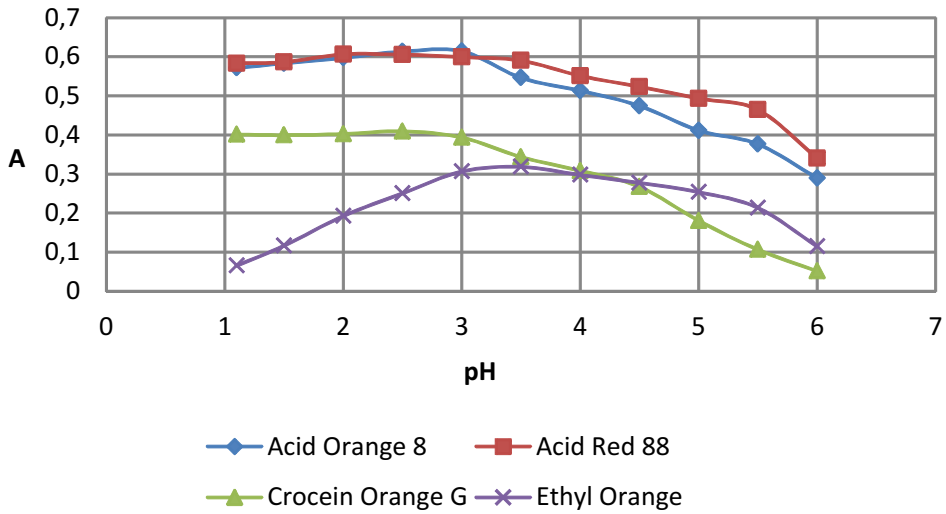

Figure 9. Remifentanil - dependence of absorbance of extracts of ion associates of remifentanil on the pH.

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