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Адрес на редакцията
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Факс (02) 987 987 4
Гл. редакпор: ✆ (02) 987 987 4
E-mail:snikolov@mbox.pharmfac.acad.bg

Address of Editorial Board
Faculty of Pharmacy
2, Dunav str., Sofia 1000
Fax (02) 987 987 4
Editor in Chief: ✆ (+359 2) 987 987 4
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ANALYTICAL STUDY OF SYNTHETIC PYRETHROID FLUMETHRIN – UV-SPECTROPHOTOMETRIC AND HPLC DETERMINATION IN VETERINARY DRUGS

Iv. Pencheva, D. Obreshkova and D. Tsvetkova

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University – Sofia

Summary. New analytical methods for quality control of Flumethrin in veterinary formulations by terms of ultraviolet spectrophotometry (UV) and high performance liquid chromatography (HPLC) are developed. Methods are validated in respect of analytical parameters: selectivity, precision, accuracy, linearity, detection limit and quantitation limit. Analytical parameter accuracy is represented by the data for the degree of recovery, which correspond to the relevant confidence interval – for the veterinary drug formulation: 94.394% ± 7.165% (UV); 100.431% ± 11.866% (HPLC); for model mixtures (UV): 95.333% ± 8.278% (I); 89.167% ± 2.041% (II); 99.583% ± 5.338% (III); for model mixtures (HPLC): 92.445% ± 2.829% (I); 93.333% ± 2.621% (II); 97.519% ± 2.012% (III). For both analytical methods the accuracy for mixtures III is better than the accuracy for mixtures I and II. In all three cases, the precision achieved by the HPLC method is better than the one achieved by UV-determination, due to the lower values of SD, RSD, SE$\bar{X}$, E (%) for all of the examined model mixtures. The influence of the factors time and temperature on the stability of Flumethrin in the analyzed veterinary product is also investigated by the terms of UV-spectrophotometry.

Key words: Flumethrin, pyrethroids, pesticides, UV-spectrophotometry, HPLC

АНАЛИТИЧНО ИЗСЛЕДВАНЕ НА СИНТЕТИЧНИЯ ПИРЕТРОИД ФЛУМЕТРИН – УВ СПЕКТРОФОТОМЕТРИЧНО И ВЕТХ ОПРЕДЕЛЯНЕ ВЪВ ВЕТЕРИНАРНИ ЛЕКАРСТВА

Ив. Пенчева, Д. Обрешкова и Д. Цветкова

Катедра по фармацевтична химия, Фармацевтичен факултет, Медицински университет – София

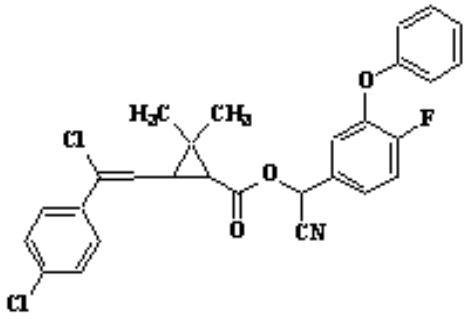
Резюме. За контрол на качеството на ветеринарни продукти, съдържащи флуметрин, са разработени нови аналитични подходи чрез прилагане на спектрофотометрия в ултравиолетовата (УВ) област и високоефективна течна хроматография (ВЕТХ). Методите са удостоверени по отношение на аналитичните параметри: селективност, прецизност, повторяемост, правилност, точност, линейност, граница на откриваемост и граница на количествено определяне. Аналитичният параметър точност е представен чрез данните за степен на възвръщане, които отговарят на съответния доверителен интервал – за ветеринарния продукт: 94.394% ± 7.165% (УВ); 100.431% ± 11.866% (ВЕТХ); за моделните смеси (УВ): 95.333% ± 8.278% (I); 89.167% ± 2.041% (II); 99.583% ± 5.338% (III); за моделните смеси (ВЕТХ): 92.445% ± 2.829% (I); 93.333% ± 2.621% (II); 97.519% ± 2.012% (III). И за двата метода точността за моделна смес III е по-добра, в сравнение с точността за моделни смеси I и II. При всички моделни смеси получената чрез ВЕТХ прецизност е по-добра от прецизността, получена чрез УВ, поради по-ниските стойности на аналитичните параметри SD, RSD, SE$\bar{X}$, E (%). Чрез УВ метод е изследвано влиянието на факторите време и температура върху стабилността на флуметрин в анализирания ветеринарен продукт.

Ключови думи: флуметрин, пиретроиди, пестициди, УВ спектрофотометрия, високоефективна течна хроматография

Introduction

During the last 60 years, society has gained many benefits from the use of pesticides to prevent disease and to increase production of food [6, 7, 9, 13]. The pyrethrines are the ones of most commonly used biocides, because of it's low toxicity, lack of persistence in the environment and possibilities for combinations with other synergistic compounds. During the last years, they find application in the veterinary medicine. Insects can be controlled by a variety of

chemicals, but in this study the discussion is limited to synthetic pyrethrins. Synthetic pyrethroid Flumethrin is 3– [2 – chloro – 2 – (4 – chlorophenyl)ethenyl] – 2.2 – dimethylcyclopropanecarboxylic acid 1 – cyano – 1 – (4 – fluoro – 3 – phenoxyphenyl) methyl ester:



Flumethrin is in use as a veterinary drug with insecticidal and acaricidal action and for prevention from ectoparasites. Pyrethroids are nerve membrane sodium channel toxins. They slow down the rate of inactivation of the sodium current elicited by membrane depolarization, thus prolonging the open time of the sodium channel. The pyrethroids which contain cyano group (Flumethrin) are generally more potent at producing membrane depolarization than non-containing agents. The interaction of pyrethroids with sodium channel complex is highly stereospecific. The slower hydrolysis of cis isomer by oxidases than trans isomer by esterases can change the toxicity of used mixture [8]. From the other side, the mechanism of neurotoxicity is related to temperature differences. Lower temperature increases the potency of pyrethroids and chlorinated derivatives to affect sodium channel function. In view of pharmacology action of pyrethroids, their applications [1, 5, 10, 11] and stability [4] we need analytical methods for quality control with greater selectivity, accuracy and repeatability. The EU and European Pharmacopoeia regulations require control and limitation for residues of pyrethroids in drugs, water, foods. The procedures include analytical methods with high performance and sensitivity [2, 3, 12]. The aim of this study is to investigate analytical parameters by UV-spectrophotometry and HPLC and to examine stability of new drug preparation containing 3.6 mg Flumethrin.

Materials and methods

I. Drug product, containing 3.6 mg Flumethrin; reference substance: Flumethrin RS; reagents: methanol, acetonitril, destilled water, o – phosphoric acid.

II. Methods for determination of Flumethrin

1. UV-spectrophotometry

Spectroscopic system: Diode array HP Spectrophotometer. *Standard preparation:* Reference substance Flumethrin RS is dissolved with solvent methanol to obtain final concentration of 0.6 μg/ml. *Blank solution:* Methanol.

2. HPLC

Chromatographic system: Liquid chromatograph Shimadzu (Japan) (LC – 10 Advp), equipped with: column RP – 18 ODS (250 mm/4.6 mm i.d./5 μm), column oven (CTO – 10 Asvp); isocratic pump (LC – 10 A); UV – VIS-detector at fixed wavelengths (SPD – 10 Avvp); 20 μl injector loop.

Chromatographic conditions: flow rate – 1.0 ml/min; column temperature – 24°C; 292 nm analytical wavelength; mobile phase: acetonitril:destilled water: o – phosphoric acid – 90:10:0.04 v/v/v. Before using, the obtained solvent mixture (mobile phase) is mixed and filtered through membrane filter with pore size 0.45 μm.

Standard preparation. An accurately weighted quantity (0.0360 g) of reference substance Flumethrin RS is dissolved in methanol in suitable size volumetric flask to obtain a known concentration of 0.0036 mg/ml. To 5.0 ml of this solution 80 ml methanol are added and the procedure for preparing of test solution is carried out.

Test preparations for UV and HPLC methods

5 tree sheets from the veterinary preparation, containing 3.6 mg Flumethrin for each are extracted with 80 ml methanol on water bath with heating at 70°C for 2 h under reflux condensor. After extraction the sample is cooled on air to room temperature and filtered in volumetric flask. The obtained solution (1) is diluted to 100.0 ml with methanol and shaked. For UV-method 1.0 ml from solution (1) is diluted to 50.0 ml in volumetric flask with methanol. For HPLC 2.0 ml from solution (1) is diluted to 10.0 ml in volumetric flask with mobile phase.

Results and discussion

I. Validation of UV-specrophotometric and HPLC methods for determination of Flumethrin

For the development of the validation procedure of UV and HPLC methods, some analytical parameters are studied such as: selectivity, precision, accuracy, linearity, detection limit and quantitation limit.

1. Selectivity (in respect of supplements) – the ability to assess unequivocally the analyte in the presence of components that may be expected to be present.

”Placebo” solutions, containing all supplements without the active substance are prepared in the same manner as test solutions. The selectivity of the applied UV-method is proved by the fact, that there is no absorption maximum on the spectrogram obtained with the respective “placebo” solution at 220 nm and 270 nm. The selectivity of the HPLC is confirmed by the fact, that on chromatograms with the respective ”placebo” preparation (tree sheets without Flumethrin) do not exist peaks with Rf responded to Flumethrin. The identity of Flumethrin is shown by the relevance between the retention times of samples of product and reference standard of Flumethrin.

2. Precision – expresses the closeness of agreement (degree of scatter) between a series of individual test measurements, obtained after applying the method repeatedly to multiple samplings of the same homogeneous sample under prescribed conditions. Repeatability (intra-assay precision) refers to apply the analytical procedure in the same laboratory, using the same analyst with the same equipment for a short period of time.

Six (6) equal homogenous samples from drug preparation containing 3.6 mg Flumethrin are analyzed separately respectively by the written UV and HPLC methods. The values for the amount of Flumethrin (C Fl.) and for the degree of recovery (R Fl.) are summarized in Table 1 (UV) and in Table 2 (HPLC). In tables, there are indicated: **N** – number of the individual measurements (1 ÷ 6); **C Fl.** – quantity of Flumethrin (mg) in drug product (with labeled of 3.6 mg Flumethrin); **R Fl.** – degree of recovery (%); $\overline{X}$ – arithmetical mean; **SD** – standard deviation; **RSD** – relative standard deviation; **SE** – standard error of arithmetical mean; $\overline{X} \div$ **t . SE** $\overline{X}$ – confidence interval; **E** – relative error.

For the estimation of the analytical parameter precision (repeatability), there is used the uncertainty of the result, which is determined by: SD, RSD and $\overline{X} \div$ **t . SE** $\overline{X}$**.** From Table 1 and Table 2 is obvious, that all of the data suit respective confidence interval (confidence possibility – P = 98.00%, coefficient of Student – t = 3.370), respectively:

– for C Fl.: 3.398 ± 0.256 (UV); 3.616 ± 0.428 (HPLC)

– for R Fl.: 94.394 ± 7.165 (UV); 100.431 ± 11.866 (HPLC).

3. Accuracy – expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Table 1. *Obtained by UV-spectrophotometry content of Flumethrin (C Fl.) and degree of recovery (R Fl.) for the investigated veterinary drug product.*

N	**C Fl. [mg]**	**R Fl. [%]**
1.	3.282	91.167
2.	3.242	90.056
3.	3.366	93.500
4.	3.631	100.861
5.	3.634	100.944
6.	3.234	89.833
$\overline{X}$ ± SD	3.398 ± 0.187	
$\overline{X}$ ± RSD [%]		94.394 ± 5.516
SD	0.187	5.207
RSD [%]	5.503	5.516
SE $\overline{X}$	0.076	2.126
t . SE $\overline{X}$	0.256	7.165
$\overline{X}$ ± t . SE $\overline{X}$	3.142 ÷ 3.654	87.229 ÷ 101.559
E [%]	2.237	2.252

Table 2. *Obtained by HPLC quantity of Flumethrin (C Fl.) and degree of recovery (R Fl.) for the examined veterinary drug formulation*

N	**C Fl. [mg]**	**R Fl. [%]**
1.	3.603	100.083
2.	3.348	93.000
3.	3.408	94.667
4.	4.032	112.000
5.	3.960	110.000
6.	3.342	92.833
$\overline{X}$ ± SD	3.616 ± 0.310	
$\overline{X}$ ± RSD [%]		100.431 ± 8.585
SD	0.310	8.622
RSD [%]	8.573	8.585
SE $\overline{X}$	0.127	3.521
t . SE $\overline{X}$	0.428	11.866
$\overline{X}$ ± t . SE $\overline{X}$	3.188 ÷ 4.044	88.565 ÷ 112.297
E [%]	3.512	3.506

The analytical parameter accuracy is performed by UV-spectrophotometry and HPLC, checking of model mixtures (I, II, III), prepared from ”placebo” solution with adding of an active substance in ratio from 80% to 120% of theoretical concentration. The content of Flumethrin, putted in mixtures is correspondingly: 3.0 mg – I (Fl. 3.0); 3.6 mg – II (Fl. 3.6); 4.0 mg – III (Fl. 4.0) (only for UV); 4.3 mg –

III (Fl. 4.3) (only for HPLC). Every sample is analyzed three times and SD and RSD are found. Experimental results for the obtained by UV and HPLC amount of Flumethrin in model mixtures: I (Fl.' 3.0); II (Fl.' 3.6); III (Fl.' 4.0) (only for UV); III (Fl.' 4.3) (only for HPLC) and the data for the degree of recovery (R): I (R 3.0); II (R 3.6); III (R 4.0) (only for UV); III (R 4.3) (only for HPLC) are included in Table 3 and Table 4.

Parameter accuracy is estimated on the base of SD; RSD; $\overline{X}$ ± t . SE $\overline{X}$ and R (presented as % obtained quantity by using a method of the external standard). From Table 3 and Table 4 is obvious, that:

1. For model mixtures I, II, III, the values for R suit respective confidence interval (confidence possibility – P = 90.00%, coefficient of Student – t = 2.92).

2. For both analytical methods, the accuracy for mixtures III is better than the accuracy for mixtures I and II.

3. In all three cases, the precision achieved by the HPLC method is better than the one achieved by UV-determination, due to the lower values of SD, RSD, SE $\overline{X}$ **,** E (%) for all of the examined model mixtures.

Table 3. *Obtained by UV-spectrophotometry content of Flumethrin (Fl.') and degree of recovery (R) for model mixtures: I (Fl.' 3.0; R 3.0); II (Fl.' 3.6; R 3.6) and III (Fl.' 4.0; R 4.0)*

N	**Fl.'3.0 [mg] [I]**	**R 3.0 [%] [I]**	**Fl.' 3.6 [mg] [II]**	**R 3.6 [%] [II]**	**Fl.' 4.0 [mg] [III]**	**R 4.0 [%] [III]**
1.	3.030	101.000	3.160	87.778	4.120	103.000
2.	2.770	92.333	3.230	89.722	3.870	96.750
3.	2.780	92.667	3.240	90.000	3.960	99.000
$\overline{X}$ SD	2.860 ± 0.147		3.210 ± 0.044		3.983 ± 0.127	
$\overline{R}$ [%] ± RSD [%]		95.333 ± 5.150		89.167 ± 1.358		99.583 ± 3.179
SD	0.147	4.910	0.044	1.211	0.127	3.166
RSD [%]	5.140	5.150	1.371	1.358	3.189	3.179
SE $\overline{X}$	0.085	2.835	0.025	0.699	0.073	1.828
t . SE	0.248	8.278	0.073	2.041	0.213	5.338
$\overline{X}$ ± t . SE $\overline{X}$	2.612 ÷ 3.108	87.055 ÷ 103.611	3.137 ÷3.283	87.126 ÷ 91.208	3.770 ÷4.196	94.245 ÷ 104.921
E [%]	2.972	2.974	0.779	0.784	1.833	1.836

Table 4. *Obtained by HPLC quantity of Flumethrin (Fl.') and degree of recovery (R) for model mixtures: I (Fl.' 3.0; R 3.0); II (Fl.' 3.6; R 3.6); III (Fl.' 4.3; R 4.3).*

N	**Fl.'3.0 [mg] [I]**	**R 3.0 [%] [I]**	**Fl.' 3.6 [mg] [II]**	**R 3.6 [%] [II]**	**Fl.' 4.3 [mg] [III]**	**R 4.3 [%] [III]**
1.	2.780	92.667	3.340	92.778	4.180	97.209
2.	2.820	94.000	3.400	94.444	4.150	96.512
3.	2.720	90.667	3.340	92.778	4.250	98.837
$\overline{X}$ SD	2.773 ± 0.050		3.360 ± 0.035		4.193 ± 0.051	
$\overline{R}$ [%] ± RSD [%]		92.445 ± 1.815		93.333 ± 1.031		97.519 ± 1.223
SD	0.050	1.678	0.035	0.962	0.051	1.193
RSD [%]	1.803	1.815	1.042	1.031	1.216	1.223
SE $\overline{X}$	0.029	0.969	0.020	0.555	0.029	0.689
t . SE	0.085	2.829	0.058	1.621	0.085	2.012
$\overline{X}$ ± t . SE $\overline{X}$	2.688 ÷ 2.858	89.616 ÷ 95.274	3.302 ÷ 3.418	91.712 ÷ 94.954	4.108 ÷ 4.278	95.507 ÷ 99.531
E [%]	1.046	1.048	0.595	0.595	0.692	0.707

Table 5. *Shovene's criterion (U) for the obtained by UV-spectrophotometry content of Flumethrin: in the investigated drug formulation (U C Fl.) and in model mixtures: I (U Fl.' 3.0), II (U Fl.' 3.6), III (U Fl.' 4.0)*

Drug product containing 3.6 mg Flumethrin		**Model mixtures of Flumethrin**			
N of sample	**U C Fl.**	**N of sample**	**U Fl.' 3.0 Mixture I**	**U Fl.' 3.6 Mixture II**	**U Fl.' 4.0 Mixture III**
1.	0.620	1.	1.156	1.136	1.079
2.	0.834				
3.	0.171	2.	0.612	0.455	0.890
4.	1.246				
5.	1.262	3.	0.544	0.682	0.181
6.	0.877				

Table 6. *Shovene's criterion (U) for the obtained by HPLC quantity of Flumethrin in the examined veterinary drug product (U C Fl.) and in model mixtures: I (U Fl.' 3.0), II (U Fl.' 3.6), III (U Fl.' 4.3)*

Drug product		**Model mixtures of Flumethrin**			
N of sample	**U C Fl.**	**N of model mixture**	**U Fl.' 3.0**	**U Fl.' 3.6**	**U Fl.' 4.3**
1.	0.042	I.	0.140	0.571	0.255
2.	0.865				
3.	0.671	II.	0.940	1.143	0.843
4.	1.342				
5.	1.110	III.	1.060	0.571	1.118
6.	0.884				

For all of the obtained by UV (Table 1) and HPLC (Table 2) results for the content of Flumethrin in every sample, it is necessary to estimate the Shovene's criterion (U), because when U for one value is higher than the rellevant standard Shovene's criterion (U St.), the data must be removed as unexpected. The results for Shovene's criterion are presented correspondingly in Table 5 (UV) and Table 6 (HPLC): for veterinary drug preparation, containing 3.6 mg Flumethrin (U C Fl.) and for model mixtures with Flumethrin: I (U Fl.' 3.0); II (U Fl.' 3.6); III (U Fl.' 4.0) (Table 5); III (U Fl.' 4.3) (Table 6).

The statistical requirements for Shovene's criterion are: U St. < 1.73 (N = 6) (for drug product) and U St. < 1.68 (N = 3) (for model mixtures I, II, III). From Table 5 there and Table 6 are obvious the relations: U C Fl. < U St.; U Fl.' 3.0 < U St.; U Fl.' 3.6 < U St.; U Fl. 4.0 ≤ U St. (Table 5); U Fl.' 4.3 ≤ U St. (Table 6), which confirm that all experimental data suit standard requirements and it isn't nessesary to remove anyone of them as unexpected.

4. Linearity – the ability of an analytical procedure to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration (amount) of analyte in the sample within a given range.

For the investigation of analytical parameter linearity, there are prepared six solutions of reference substance Flumethrin RS with decreasing concentration of drug and they are analyzed respectively by the written UV and HPLC methods. The proportional accordance between the absorption (A), measured in absorption unites (AU) and concentration (C) in μg/ml is found and the data are shown in Fig. 1. For HPLC the dependences concentration (C) in μg/ml/peak height (H) are presented in Fig 2. The results are putted into linearity regression analysis and the coefficient of regression (R) is calculated. RSD is in the normal limits. There is no mistake of the linear proportion and no mistake of the dependence of the other constituents of the mixture.

5. Detection limit (LOD) = C_{LOD} = C_{min} and quantitation limit (LOQ). The detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated as an exact value. The quantitation limit is the lowest concentration of analyte in a sample that can be determined with suitable precision and accuracy under the stated experimental conditions.

For the applied UV-method: C_{LOD} = 0.03 μg/ml; C_{LOQ} = 0.3 μg/ml

For HPLC: C_{LOD} = 0.6 μg/ml; C_{LOQ} = 6.0 μg/ml

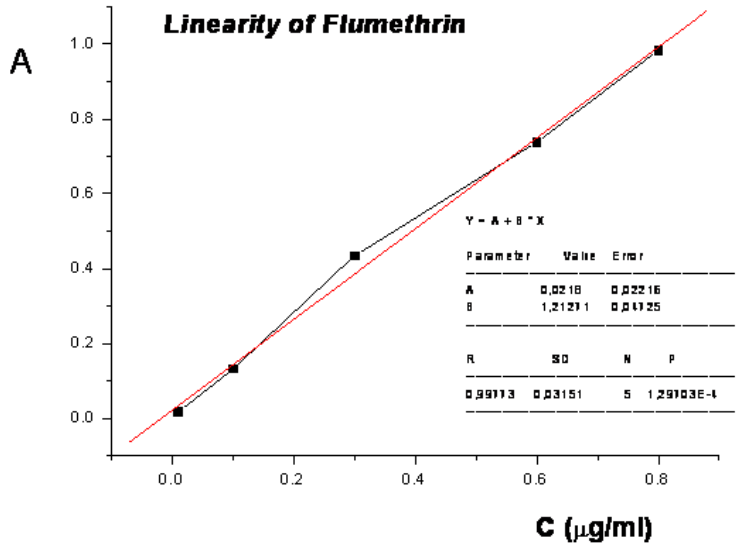


Fig. 1. *Linearity of Flumethrin, obtained by UV-spectrophotometry*

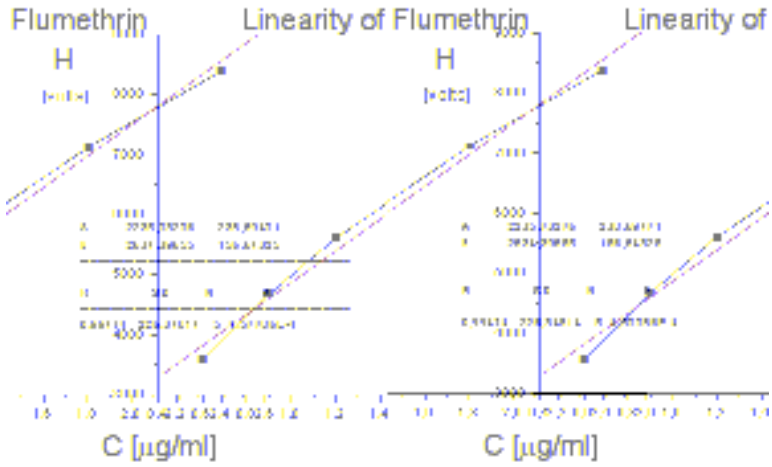


Fig. 2*. Linearity of Flumethrin, obtained by HPLC*

IV. Stability of Flumethrin

For the investigation on stability of Flumethrin the test solution is analyzed by UV-spectrophotometry for 120 h at about 25°C. From the 6th hour, it is proved the beginning of the change of the spectral curve. It is observed hypochromic shift at 220 nm and 270 nm and the values of absorption are decreased. Between analysis, the samples are stored at 8°C. The % of obtained quantity has been decreased too. The results show that the samples are in tolerance for 18 h (91.06%). Extraordinary increasing of temperature by 3°C led to hyperchromic shift of spectral curve, which is an indication for decomposition. The results are shown in the Table 7, where A_1 and A_2 are absorption values at 220 nm and 270 nm.

Table 7. *Stability of Flumethrin in drug preparation in dependence of time and temperature analyzed by UV-method*

Time [h]	**A_1 [AU] ± 0.0001 SD**	**A_2 [AU] ± 0.0001 SD**	**t [°C]**	**Obtained quantity of Flumethrin [%]**
0	2.304	0.403	24	100.00
6	2.227	0.374	26	92.83
18	1.605	0.367	25	91.06
24	1.513	0.284	25	70.47
42	1.513	0.273	25	67.74
48	2.219	0.300	28	74.42
66	2.172	0.294	25	72.98
120	2.124	0.307	25	74.24

Conclusion

An analytical procedure for the determination by UV and HPLC methods of pyrethroide pesticide Flumethrin is developed. The applied analytical methods are found to be selective, reproducible and accurate in appointed linear intervals. All of the obtained by both methods results correspond to the relevant confidence interval. The comparison of results obtained from validation of methods show, that the most important parameters are accuracy and selectivity. They must be proved every time at equal conditions. Obtained results are important for validation of producing and storage procedures of drugs containing Flumethrin.

References

1. A n a d o n , A. et al. Effects of flumethrin on hepatic drug-metabolizing enzyme antipyrine disposition in rats. – Toxicol. Appl. Pharmacol., **132**, 1995, № 1, 14-18.
2. B i s s a c o t , D. Z. et I. Vassilieff. HPLC determination of Flumethrin, Deltamethrin, Cypermethrin and Cyhalothrin residues in the milk and blood of lactating dairy cows. – J. Anal. Toxicol., **21**, 1997, № 5, 397-402.
3. B i s s a c o t , D. Z. et I. Vassilieff. Pyrethroid residues in milk and blood of dairy cows follow topical applications. – Vet. Hum. Toxicol., **39**, 1997, № 1, 6-8.
4. K o r t a , E. et al. Study of the degradation products of bromopropylate, chlordimeform, coumaphos, cymiazole, flumethrin and taufluvalinate in aqueous media. – Talanta, **52**, 2000, № 2, 169-180.
5. K o r t a , E. et al. Study of acaricide stability in honey. Characterization of amitraz degradation products in honey and beeswax. – J. Agric. Food. Chem., **49**, 2001, № 12, 5835-5842.
6. M a t t i o l i , R. C., J. Jaitner et M. Bah. Efficiency and cost of strategic use of acaricide for tick control in N'Dama cattle in the Gambia. – Med. Vet. Entomol., **13**, 1999, № 1, 33-40.
7. M e k o n n e n , S. Efficacy of Flumethrin 1% pour-on against ticks on cattle under field conditions in Ethiopia. – J. Vet. Res., **67**, 2000, № 4, 235-237.
8. M i l l e r , R. J., R. B. Davey et J. E. George. Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican Boophilus microplus (Acari: Ixodidae). – J. Med. Entomol., **36**, 1999, № 5, 533-538.
9. N a u m a n n , K. Research into fluorinated pyrethroid alcohols: An episode in the history of pyrethroid discovery. – Pestic. Sci., **52**, 1998, № 1, 3-20.
10. R u u g t , G. S. F. et J. Van Den Bercken. Action of pyrethroids on a nerve-muscle preparation of the clawed frog, Xenopus laevis. Pestic. – Biochem. Physiol., **25**, 1986, № 2, 176-187.
11. S e m p l e , K. T. et al. Impact of synthetic pyrethroid-sheep dip on the indigenous microflora of animal slurries. – FEMS Microbiol. Lett., **190**, 2000, № 2, 255-260.
12. S z e r l e t i c s – T u r i, M. Determination and control of bee-acaricide flumethrin in honey and beewax. – Acta Alimentaria, **28**, 1999, № 1, 85-94.
13. W i l l i a m s , D. L. A veterinary approach to the European honey bee (Apis mellifera). – Vet. J., **160**, 2000, № 1 61-73.

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✉ **Address for correspondence:**
Assist. Prof. Ivanka Petkova Pencheva, Ph. D.
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
Medical University
2 Dunav Str.
1000 Sofia
✆ +35929236503
🖷 +35929879874
e-mail: ivatibi@yahoo.com

✉ **Адрес за кореспонденция**
Доц. д-р Иванка Петкова Пенчева
Катедра по фармацевтична химия
Фармацевтичен факултет
Медицински университет
ул. "Дунав" № 2
1000 София
✆ +35929236503
🖷 +35929879874
e-mail: ivatibi@yahoo.com