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## CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

# DETERMINATION OF LAMBDA-CYHALOTHRINE IN MICRO-INCAPSULATED INSECTICIDAL COMPOSITIONS

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*The determination of the title compound in the model microencapsulated insecticidal tools is considered, as well as the ability to conduct analysis in mixtures with other encapsulated means on the basis of a pyrethroid (cypermethrin, deltamethrin, tetramethrin, permethrin, phenothrin, transfluthrin) and phosphorus-containing derived insecticidal substances (chlorpyrifos). The conditions of direct analytical determination and the stage of sample preparation with the previously described methods are adapted. The content of lambda-cyhalothrin together with cypermethrin in microencapsulated compositions is determined by spectrophotometric method taking into account the absorption of trace contaminants – technical substances and building blocks of microcapsules (phospholipids). RP HPLC method shows the possibility of separate determination of lambda-cyhalothrin and several pyrethroids (except for cypermethrin), and chlorpyrifos, in the hypothetical compositions in a joint encapsulating order to obtain a highly active insecticide that enable overcoming the resistance of insects to a number of inactive substances.*

***Keywords:*** *lambda-cyhalothrin, insecticid, microcapsules, deltamethrin, cypermethrin, permethrin, chlorpyrifos, determination, UV, RP HPLC, «pest control».*

The microencapsulated form [1] of pesticides inclusion in the structure of compositions is one of the most successful from the viewpoint of user-friendliness, stability and safety. This form is applied for medical disinsection [2–4].

The range of encapsulated insecticidal substances used for "pest control" constantly ex-tends including pyrethroid [5–8] and organophosphorus [5, 9, 10] derivatives, as well as carbamates [4]. Microencapsulated insecticidal substances often surpass other preparative forms in such indicators as residual action, slight smell (suppression of repellent properties), low toxicity for warm-blooded animals and human beings. Only the high cost and technological features of producing such forms are limiting factors for their more widespread manufacturing application by most producers of means for "pest control".

Since the continuous use of insecticidal substances leads over time to the formation of popu-lations of insects resistant to certain active ingredients of insecticides, it is necessary to consider the possibility of combining several substances of different classes or using predeterminedly more active derivatives. For example, it was recommended to use a mixture of microencapsulated forms (1:1) based on chlorpyriphos (a phosphorus-containing insecticide) and cypermethrin (a pyrethroid insecticide), because the composition with this ratio shows synergetic effect when testing on German cockroaches [11]. In other cases producers of insecticides offer more active encapsulated sub-stances, for example, those based on such powerful pyrethroid insecticides as deltametrin [6] and a lambda-cyhalothrin [7]. However, producing a microencapsulated preparation with a highly active pyrethroid substance as an active ingredient in concentrations higher than 5% is a serious problem in case of designing the microcapsule envelopes from the most ecofriendly and safe natural materials based on phospholipids (liposomes). Recently, a method for preparing compositions was suggested, which allows including in the structure simultaneously cypermethrin and lambda-cyhalothrin with a large prevalence of the latter [12]. This resulted in the necessity of controlling the content of the initial substances in the test microencapsulated compositions. The role of cypermethrin having lower biological activity is reduced to stabilization of encapsulation of the more active pyrethroid insecticide – lambda-cyhalothrin.

### Results and Discussion

A spectrophotometric method has been suggested before for the determination of cyperme-thrin microencapsulated in liposomes with the content of the active ingredient of 10% [13]. Using this approach as a base we tried to adapt this convenient and rapid method requiring no complex chromatographic equipment for the combined determination of encapsulated cypermethrin

and lambda-cyhalothrin. The absorption spectra of cypermethrin and lambda-cyhalothrin (solutions are prepared from state standard samples, hereafter referred to as SSS) are presented in Figure 1 (●, unmarked).

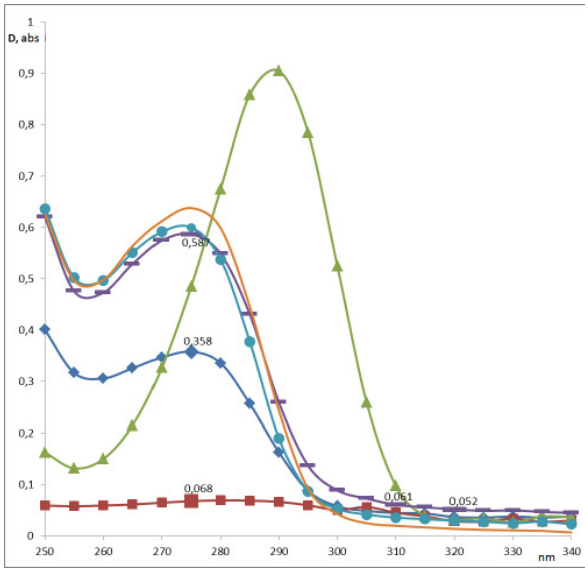


**Fig. 1.** Optical spectra of solutions in the UV range (250–340 nm):
♦ – eluate (5 ml) of the major component after TLC separation ($R_f$ 0.6–0.8) of commercially pure lambda-cyhalothrin;
■ – eluate (5 ml) of trace impurities after TLC separation ($R_f$ 0.1–0.5) of commercially pure lambda-cyhalothrin;
● – 0.127 mg/ml solution (prepared from SSS) of cypermethrin in isopropanol;
▬ – solution containing 0.13% of the preparation (0.013% of the sum of pyrethroids)
of the model microencapsulated lambda-cyhalothrin – cypermethrin composition (8:2) in isopropanol;
▲ – 0.057 mg/ml solution of chlorpyriphos in isopropanol prepared from SSS;
unmarked – 0.159 mg/ml solution of lambda-cyhalothrin in isopropanol prepared from SSS.

In order to establish the influence of impurities on the spectrophotometric determination of a substance/preparation, lambda-cyhalothrin was subjected to a test with preliminary separation on Sorbfil plates. The systems of eluents tested by us for planar chromatography (Table 1) allow rec-ommending hexane-chloroform mixture (4 : 1) for the separation of impurities and the major com-ponent. The spectrophotometric analysis of ethanolic eluates of spots from the plates with the major component ($R_f$ 0.6–0.8) and impurity phases ($R_f$ 0.1–0.5 in total) carried out according to the general scheme [14] shows (Figure 1) a spectrum similar to that of cypermethrin and is in agreement with previously obtained spectral data [13, 15, 16]. Other eluents suggested for the analysis of phosphorus-containing insecticides [17, p. 55] and cypermethrin [17, p. 61] on Silufol plates do not provide separation of a technical preparation of lambda-cyhalothrin into components when using Sorbfil plates.

It should be noted that according to RP-HPLC the ratio [content of trace impurities in the standard lambda-cyhalothrin solution (from SSS) (peaks No. 1–3, Figure 2a) / content of the major component (peak No. 5)] is 18:82, which almost coincides with the results of separate spectropho-tometric determination of commercial purity substance produced in India with the label claim of the major component not less than 98%: the spectral data indicate that the ratio impurity / main compo-nent at 275 nm is 16:84 (0.068:0.358, see Figure 1). The content of lambda-cyhalothrin in the com-mercial purity substance (X, %) was calculated with the use of the concentration characteristics and the value of molar absorptivity at 275 nm according to formula (1):

$$X = \frac{D^{275} \cdot M.w. \cdot 0.050 \cdot 0.005 \cdot 100\%}{m \cdot 0.0006 \cdot \varepsilon^{275}} \qquad (1)$$

where

$D^{275}$ is the optical density of the analyzed extract of the major fraction ($R_f$ 0.6–0.8) at 275 nm;

*M.w.* is the molecular weight of lambda-cyhalothrin (499.9 g/mol);

0.050 is the volume of chloroform, in which the substance was dissolved, l;

*m* is the mass of the substance, g;

$\varepsilon^{275}$ is the molar extinction coefficient (2000 l/(mol·cm) of lambda-cyhalothrin (at 275 nm) [14]);

0.005 is the volume of the ethanol solution, by which the spot is eluted from the plate, l;

0.0006 is the volume of the basic solution (in chloroform), which is applied onto the plate, l.

**Table 1.** Eluents and $R_f$ values for TLC of lambda-cyhalothrin and cypermethrin

| Compound | Eluent | $R_f$ |
|---|---|---|
| Lambda-cyhalothrin* | $CHCl_3$ – $CCl_4$ (2:1) | 1.0 |
| | $CHCl_3$ – $CCl_4$ (1:1) | 1.0 |
| | $CCl_4$ | 1.0 |
| | Hexane | 0 |
| | Hexane – acetone (4:1) | 1.0<br>0.95 |
| | Hexane – CHCl3 (4:1) | 0.6–0.8 (major comp.)<br>0.4–0.5 (imp. phase)<br>0.3–0.4 (imp. phase)<br>0.1–0.2 (imp. phase) |
| Cypermethrin** | $CHCl_3$ – $CCl_4$ (1:1) | 0.5 [14] |
| | Hexane – acetone (4:1) | 0.44 [17, c. 61] |
| Deltamethrin** | Hexane – acetone (4:1) | 0.44 [17, c. 61] |
| | $CHCl_3$ – $CCl_4$ (1:1) | 0.76 |

* for Sorbfil plates
** for Silufol plates

In the analysis conditions [$D^{275}$ = 0.378; m = 0.0377; Figure 1 (♦)] the established content of lambda-cyhalothrin in the commercial purity preparation is close to that determined by RP-HPLC (Table 2).

The literature data on the spectral characteristics of homologs of the pyrethroid series: lambda-cyhalothrin ($\lambda_{max}$ = 275 nm, $\varepsilon^{275}$ = 2000 l/(mol·cm) [14]) and cypermethrin ($\lambda_{max}$ = 277 nm, $\varepsilon^{277}$ = 1960 l/(mol·cm) [14, 16] and $\lambda_{max}$ = 280 nm, $\varepsilon^{280}$ = 1790 l/(mol·cm) [15]) in ethanol, as well as our own measurements (Figure 1) show similarity of their spectral parameters. Thus, the general technique for the analysis of the model microencapsulated composition can be corrected on the basis of additive contributions of molar extinction coefficient of lambda-cyhalothrin and cypermethrin with the ratio of components equal to 8:2 at 275 nm:

$$\varepsilon^{\Sigma 275} = (0.8 \cdot \varepsilon^{lambda}) + (0.2 \cdot \varepsilon^{cyper}) \quad (2)$$

where

$\varepsilon^{lambda}$ is the value of the molar extinction coefficient (2000 l/(mol·cm) of lambda-cyhalothrin (275 nm) [14]);

$\varepsilon^{cyper}$ is the value of the molar extinction coefficient of cypermethrin (275 nm) calculated as the arithmetic average for the series of three solutions prepared from SSS (1920 l/(mol·cm)).

Since liposomal forms of insecticidal preparations are decomposed upon dilution by alcohols [17, p. 70], it should be considered in the course of the analysis, along with the absorption of trace impurities of a pyrethroid insecticide, the additional absorption of total egg lipids in the chosen area. This task has been successfully solved before in the analysis of cypermethrin by the introduction of a correction taking into account the total content of impurity forms (measurement of optical density at 310 and 320 nm) [13]. A similar compensation correction was also introduced in the analysis of other disinfective compositions [18]. Taking into account the above, the general technique for the analysis of the model microencapsulated composition takes the form presented in Experimental part. Calculation of the total content of pyrethroid insecticides is performed according to formula (3):

$$X = \frac{\left[D^{275} - \left(D^{310} + 3 \cdot \left(D^{310} - D^{320}\right)\right)\right] \cdot M.w. \cdot 0.015 \cdot 0.010 \cdot 100\%}{m \cdot 0.002 \cdot \varepsilon^{\Sigma 275}} \quad (3)$$

where

$D^{275}$, $D^{310}$ and $D^{320}$ are the optical densities of the analyzed solution of the microencapsulated insec-ticide at 275, 310 and 320 nm;

**Table 2.** Results of determination of the content of lambda-cyhalothrin in the commercial purity preparation and in a model insecticidal composition in the presence of cypermethrin

| Insecticidal composition | Introduced | Found | Method |
|---|---|---|---|
| Commercial purity lambda-cyhalothrin (India) | At least 98% | 99.2±0.4% | RP-HPLC |
| | | | TLC+spectrophotometry |
| Model microencapsulated substance: lambda-cyhalothrin – cypermethrin (8:2) | 10.0±0.2% | 9.5±0.2% | spectrophotometry |

*M.w.* is the average molecular weight of the pyrethroid composition (calculated similarly to formula (2)) of lambda-cyhalothrin and a cypermethrin (482 g/mol);

0.015 is the volume of isopropanol, in which the sample was dissolved, l;

*m* is the mass of the sample, g;

$\varepsilon^{\Sigma 275}$ is the value of molar extinction coefficient according to formula (2) for the mixture of cyper-methrin and lambda-cyhalothrin (1920 l/(mol·cm));

0.010 is the volume of the densimeter, into which an aliquot for dilution is transferred, l;

0.002 is the volume of the aliquot of the initial solution for dilution, l.

In the analysis conditions ($D^{275}$ = 0.587, D310 = 0.061, $D^{320}$ = 0.052; m = 0.09905; Figure 1 (—)) the found content of the sum of pyrethroids in the commercial purity preparation is 9.5% (tab. 2).

Alternatively, we considered the possibility of carrying out the analysis to determine the content of lambda-cyhalothrin by RP-HPLC in the microencapsulated preparations on the basis of phospholipids preserving the sample preparation described above for the spectrophotometric method. The presence of phospholipids does not hinder the identification of insecticidal substances. However, the variation of the mobile phase polarity prevents from carrying out separate detection of lambda-cyhalothrin and

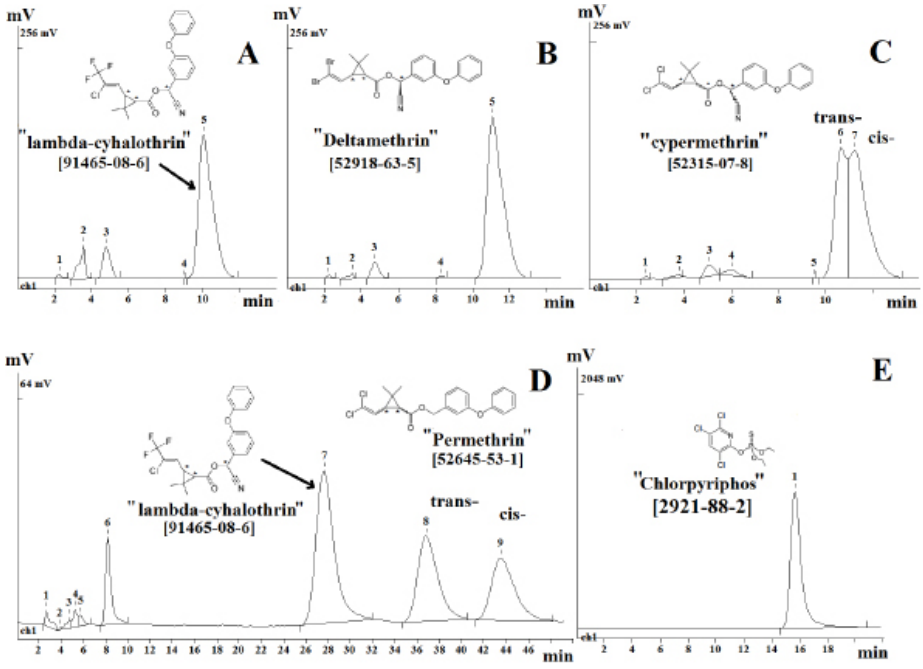


**Fig. 2.** Chromatograms of model solutions of pyrethroid insecticides:
0.99 mg/ml of "Lambda-cyhalothrin" prepared from SSS (A); 0.99 mg/ml of "Deltametrin" (from SSS) (B);
0.96 mg/ml of "Cypermethrin" (from SSS) (C).
$CH_3CN – H_2O – CH_3COOH$ mixture (80 : 20: 1), 0.5 ml/min, λ = 280 nm.
Mixture of synthetic pyrethroids lambda-cyhalothrin – 0.49 mg/ml and "Permethrin" – 0.48 mg/ml (from SSS) (D);
0.99 mg/ml of "Chlorpyriphos" (from SSS) (E).
$CH_3CN – H_2O – CH_3COOH$ mixture (70 : 30: 0.5), 0.5 ml/min, λ = 280 nm

cypermethrin because of close retention times (Figure 2a, c; 3). At the same time it is possible to carry out separate determination of lambda-cyhalothrin, chlorpyriphos, deltamethrin, permethrin and other pyrethroid insecticides: tetramethrin, transfluthrin and phenothrin (Figure 2d, e; 3) in a more polar eluent in the isocratic mode. (These insecticides are used or perhaps will be used in combination to enhance the action of the microencapsulated compositions.) It should be noted that, in addition to the close retention times of cypermethrin, lambda-cyhalothrin and deltamethrin (26–31 min, see Figure 3), it is difficult to include the latter two individual substances in the composition of liposomes in high concentration (higher than 3%). However, in the presence of cypermethrin it is possible to stabilize a considerably larger (than 3–5%) amount of highly active deltametrin and lambda-cyhalothrin encapsulated in phospholipid microcapsules [12].

Apparently, the analysis for deltamethrin as a part of the microencapsulated composition with simultaneous presence of cypermethrin as a solubilizer in view of spectral characteristics close to lambda-cyhalothrin (in case of deltamethrin $\lambda_{max}$ = 275 nm, $\varepsilon^{275}$ = 3040 l/(mol·cm) [16]) can also be carried out spectrophotometrically (summarily) with the use of formula (3) considering the molar ex-

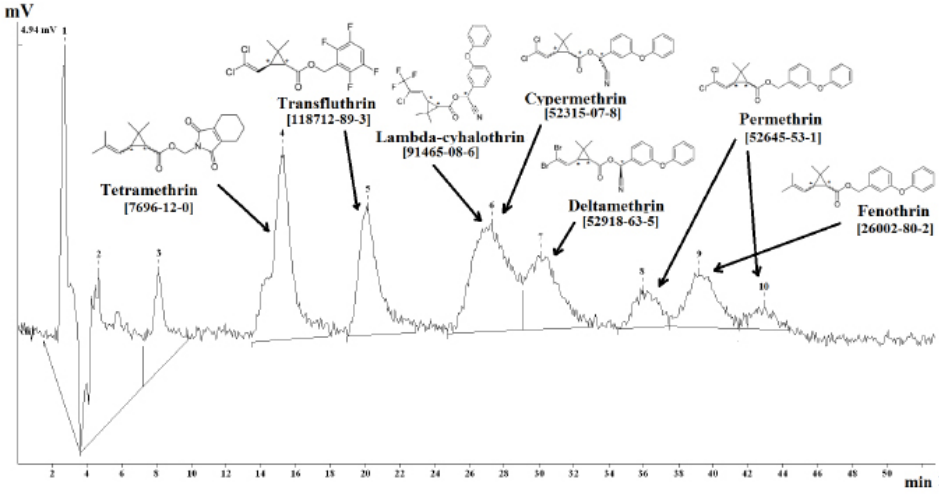


**Fig. 3.** Chromatogram of a model mixture of pyrethroid insecticidal compounds: "Tetramethrin" – 50.0 μg/ml; "Transfluthrin" – 49.0 μg/ml; "Lambda-cyhalothrin" – 9.93 μg/ml; "Cypermethrin" – 9.60 μg/ml; "Deltamethrin" – 9.95 μg/ml; "Permethrin" – 9.50 μg/ml and "Fenothrin" – 10.0 μg/ml. $CH_3CN$ – $H_2O$ – $CH_3COOH$ mixture (70 : 30 : 0.5), 0.5 ml/min, λ = 280 nm.

tinction coefficient for the total composition (see Formula (2)) or separately by RP-HPLC in the isocratic mode with the use of a more polar eluent: $CH_3CN$ – $H_2O$ – AcOH (70 : 30 : 0.5) at 280 nm.

In case of combined application of compositions based on chlorpyriphos and a pyrethroid insecticide (cypermethrin, deltamethrin, lambda-cyhalothrin) it is not possible to use the spectro-photometric method because of the high spectral characteristics of chlorpyriphos ($\lambda_{max}$ = 290 nanometers, $\varepsilon^{290}$ = 5600 l/(mol·cm) [16]) in the range of pyrethroids detection (Figure 1 (▲)). However, using $CH_3CN$ – $H_2O$ – AcOH mixture (70 : 30 : 0.5) as an eluent at 280 nm allows to determine the components of such possible microencapsulated compositions by RP-HPLC.

## Experimental

The following analytical standards were used for carrying out the investigations: "Perme-thrin" of 99.5% purity (SSS 7715-99, "Blok-1" Research and Production Corporation, hereafter re-ferred to as "Blok-1" RPC, Russia), "Cypermethrin" of 96.4% purity (SSS 7736-99, "Blok-1" RPC, Russia), "Deltamethrin" of 98.1% purity (SSS 7500-98, "Blok-1" RPC, Russia), "Lambda-cyhalothrin" of 97.5% purity (SSS 7732-99, "Blok-1" RPC, Russia), and also some other insecticidal substances produced in China and India with the declared content of the main component not lower than 96%.

Isopropanol (reagent grade, GOST 18300-87), chloroform (reagent grade, TU 6-09-06-4263), hexane (reagent grade, TU 6-09-3375-78), carbon tetrachloride (reagent grade, GOST 20288-74), acetic acid (reagent grade, GOST 61-75), distilled water (GOST 6709-72), acetonitrile (for HPCL, "Panreac", Spain) and ethanol (for HPLC, "J.T.BAIKER® BAKER ANALYZEDTM", Holland) were used without preliminary purification. For the preparation of the model microencap-sulated bait egg lipids extracted from egg powder (GOST 2858-82) were used according to the method described below.

For planar chromatography Sorbfil plates on aluminum foil (TU 26-11-17-89, PTSKH-AF-A-UF brand) of 10×15 cm size were used.

The spectrophotometric analysis of samples was carried out on an SF-46 spectrophotometer ("LOMO", USSR) in the range of 250–340 nm in quartz cells with a length of absorbing layer of 1 cm using ethanol (isopropanol) as a comparison solution.

HPLC in combination with UV detection was carried out on a Waters 490 chromatograph (Waters Ltd., Watford, UK) equipped with an Altex-110A pump, a Rheodyne injector with a loop volume of 20 μl, a UV detector (model 490) with variable wavelength. A 4.0×150 mm column made of stainless steel was used. The column was filled with Separon SGX C18 Super (RP-S) with 5 μm grains ("Elsiko", Russia). The mobile phase was acetonitrile – water – acetic acid (70 : 30 : 0.5 and 80 : 20 : 1) with a flow rate of 0.5 ml/min (previously degassed by means of an ultrasonic installation). Detection was carried out at 280 nm at room temperature. Chromatograms were recorded by means of Multikhrom program (Ampersand Ltd, version 1.52i, Russia).

In the course of the research, an original microencapsulated composition based on egg lipids containing 8% of the title compound and 2% of cypermethrin (as a solubilizer) was tested for the content of active ingredients (totally). Egg lipids (totally) were preliminarily extracted with chloroform (0.5 l) from 150 g of egg powder on a magnetic

stirring for 2 h. Then the solid phase was filtered off with the use of a filtering bottom funnel (porosity 100). The filtrate was collected and evaporated on a rotary evaporator in vacuo at 35 °C. Yield: 30–32 g (~ 20%).

Preparation of the model microencapsulated substance. Samples of lambda-cyhalothrin (8 g) and cypermethrin (2 g) in 17 cm3 of isopropanol were heated in a water bath in a flask at periodic mechanical stirring until dissolution was complete. The obtained solution of the mixture of insecti-cides was poured to 30 g of egg lipids. The resulting mixture was thoroughly stirred until a homo-geneous mass was obtained. A 250 $cm^3$ glass containing 45 $cm^3$ of distilled water was equipped with a digital stirrer with the top installation of AMTAST INC MH 2E (USA) with a microbladed impeller. The mass of lipids with the insecticide mixture was added in small portions under stirring (2000–2200 rpm). The stirring was continued for 0.5 h. The obtained composition was stored at room temperature in a flask with a ground stopper.

Determination of lambda-cyhalothrin in commercially pure preparation. The basic solution (0.6 $cm^3$, containing 37.7 mg of the commercially pure substance in 50 $cm^3$ of chloroform) was taken with a glass pipette and applied in small portions (every time the solvent was dried) on the starting line of a Sorbfil plate located at 2 cm from the edge in the form of a strip 4–5 cm long. After drying in the air for 0.1–0.2 h the plate was placed in a chromatography chamber preliminarily filled with the mobile phase (see Table 1). After the front of the solvent rose by ~ 12 cm, the separation was stopped, and the plate was dried in the air for 0.5 h. Then the observed spots were outlined with a graphite pencil in UV light. The plate part with the outlined spot was cut out, placed in a 10 cm3 centrifugal test tube, and 5 ml of ethanol was added for the spots of the major component ($R_f$ 0.6–0.8) and those of the impurities ($R_f$ 0.1–0.5 totally). The spots of the main component and of the impurities were washed away carefully from the plate under rotation of the test tube in a support by means of light rotary motions carried out manually (in order to avoid washing off of parts of the sorbent into the solution). Then the ethanolic eluate was carefully poured into a cell with the ab-sorbing layer thickness of 1 cm. The solution was analyzed photometrically in the range of 250–340 nm with a step of 5 nanometers (Figure 1 (♦, ■)). Alternatively, the content of lambda-cyhalothrin in a commercially pure preparation was determined by RP-HPLC. For this purpose the basic solution of the substance diluted by a factor of 7 was chromatographed with isopropanol. The results of the determination are presented in Table 2.

General analysis technique for model microencapsulated composition. A 70–100 mg sample of the microencapsulated model preparation was prepared in a 25 cm3 test tube with a ground stopper. (The sample should be vigorously shaken manually before weighing for the analysis or stirred by means of a stirrer for 3 min.) Isopropanol (15 $cm^3$) was added. The test tube was closed by a stopper and vigorously shaken for 3 min. Then 2 $cm^3$ of the mixture was pipetted and transferred to a 10 $cm^3$ densimeter. The solution in the densimeter was diluted by isopropanol to 10 $cm^3$ and stirred. Then spectrophotometric analysis of the obtained solution was carried out in a cell with the thickness of the absorbing layer of 1 cm at 275, 310 and 320 nm.

The sample preparation for the determination of the mixture of lambda-cyhalothrin and a cypermethrin in the model microencapsulated composition is carried out similarly to the sample preparation for the analysis of the microencapsulated preparations based on cypermethrin [13] and a chlorpyriphos [19, c. 92].

## Conclusions

The presented article describes methods for the determination of model microencapsulated insecticides containing the title compound (lambda-cyhalothrin) as well as the possibility of carrying out the analysis of mixtures with other encapsulated insecticides based on pyrethroid (cypermethrin, deltamethrin, tetramethrin, permethrin, fenothrin, transfluthrin) and phosphorus-containing insecticidal substances (chlorpyriphos). Conditions were adapted for carrying out the analytical determination as such and for the sample preparation with the use of methods described before. The determination of lambda-cyhalothrin in combination with cypermethrin in microencapsulated compositions by the spectrophotometric method considering the absorption of trace impurities of commercially pure substances and the absorption of construction blocks of microcapsules (phospholipids) was realized. The use of RP-HPLC showed the possibility of separate determination of lambda-cyhalothrin and a number of pyrethroids (except for a cypermethrin), as well as chlorpyriphos, in hypothetical compositions at joint encapsulation for the purpose of obtaining highly active insecticidal preparations allowing to overcome the resistance of insects to a number of low-active substances

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