EVALUATION OF THE PERFORMANCE OF QUANTITATIVE IR AND UV SPECTROPHOTOMETRIC METHODS WITHOUT PRELIMINARY SEPARATION IN THE ANALYSIS OF SOME PLANT PROTECTIVES, USING MATHEMATICAL STATISTICAL METHODS

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Summary

It was found that in the IR spectrophotometric quantitative analysis of the multicomponent systems studied (2–4 components) without preliminary separation the residual standard deviation of absorbance varied between 0.02 and 0.005 (most frequently between 0.006 and 0.009, depending on the absorbance value measured). The 95% probability confidence interval of concentration estimation in percentage of the concentration measured was generally 1–10%. Components present at a concentration of at least 10% could be mostly measured at a relative error of ±5%.

Principle

In this paper IR and UV spectrophotometric methods developed for the determination of the active ingredients of some plant protectives without preliminary separation will be described, and the performance of the methods will be estimated by mathematical statistics [1].

During our work the IR and UV transmission characteristics of the constituents of plant protectives have been studied, and comparing them with one another, an absorption band has been selected in the IR spectrum of the active ingredient to be determined, in the wavelength region of which the accompanying components did not reveal specific absorption. Using calibration mixtures similar in composition to that of the samples to be analyzed, the absorbance—concentration relationship has been determined at the selected band of the active ingredient in various plant protectives, and the concentration of the active ingredient was estimated in several samples. Mathematical statistical parameters characteristic of the method developed, and the error of concentration determination were calculated.

Mathematical evaluation of measuring methods

For the first of our analytical methods to be described we show in detail the evaluation of our measuring results in a tabular form (Table 1). The tables contain the concentration (x) and the absorbance (A) values (in the formulas y value) of the members of the calibration series, and the absorbance values measured for the unknown samples. Next, the equation of the linear calibration curve ($y=a+bx$) calculated by the method of least squares is given.

Parameters of the calibration line *were estimated* from the following expressions:

$$b=\frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sum_{i=1}^{n}(x_i-\bar{x})^2} \qquad a=\bar{y}-b\bar{x}\,.$$

For the statistical characterization of the parameters of the calibration line the following data were calculated:

— *standard deviation of the slope* of the calibration line

$$S_b=\sqrt{\frac{S_{res}^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$

— *standard deviation of the intercept* of the calibration line

$$S_a=\sqrt{S_{res}^2\left(\frac{1}{n}+\frac{\bar{x}}{\sum_{i=1}^{n}(x_i-\bar{x})^2}\right)}$$

— *residual standard deviation:* estimation of the standard deviation of the error of analytical signal generation (A and y, resp.), which is the square rootdrawn from the average of the squares of distances d_i in y direction ($Y_{xi}-y_i$), standard deviation estimated on the basis of all the signals measured. d_i is the value of the error of absorbance measurement, belonging to the i-th measurement.

$$s_{res}=\sqrt{\frac{\sum_{i=1}^{n}(Y_{xi}-y_i)^2}{n-2}}=\sqrt{\frac{\sum_{i=1}^{n}(bx_i+a-y_i)^2}{n-2}}$$

Remark: of n data measured two were used for the estimation of a and b, thus, of n data only $n-2$ are independent.

Table 1

CALIBRATION:	CONCENTRATION (X)	ABSORBANCE (A), respectively (Y)		
	5.000	0.060	0.063	0.065
	10.000	0.121	0.125	0.130
	20.000	0.260	0.250	0.253
	30.000	0.375	0.377	0.381
	40.000	0.498	0.500	0.508
	50.000	0.628	0.635	0.637
SAMPLES:				
1.		0.083	0.080	0.094
2.		0.448	0.440	0.450
3.		0.468	0.460	0.462
4.		0.469	0.471	0.475

CALIBRATION CURVE: A = −0.000541 + 0.012636 * X

Standard deviation of the intercept: .9315E-02
...of the slope: .6195E-04
residual standard deviation: .4185E-02
...of mean X value: .9864E-03

				95% CONFIDENCE INTERVAL	
X	A	ARTS%	XRTS%	ALKI	AUKI
5.0000	0.0626	2.59	2.57	0.0595	0.0658
7.2500	0.0911	1.66	1.65	0.0881	0.0940
9.5000	0.1195	1.18	1.18	0.1167	0.1223
11.7500	0.1479	0.89	0.89	0.1454	0.1505
14.0000	0.1764	0.70	0.69	0.1740	0.1788
16.2500	0.2048	0.56	0.56	0.2025	0.2071
18.5000	0.2332	0.47	0.46	0.2311	0.2354
20.7500	0.2617	0.40	0.39	0.2596	0.2637
23.0000	0.2901	0.35	0.34	0.2881	0.2921
25.2500	0.3185	0.31	0.31	0.3166	0.3205
27.5000	0.3469	0.29	0.29	0.3450	0.3489
29.7500	0.3754	0.27	0.27	0.3734	0.3774
32.0000	0.4038	0.26	0.26	0.4017	0.4059
34.2500	0.4322	0.26	0.26	0.4301	0.4344
36.5000	0.4607	0.26	0.26	0.4583	0.4630
38.7500	0.4891	0.26	0.26	0.4866	0.4916
41.0000	0.5175	0.26	0.26	0.5149	0.5202
43.2500	0.5460	0.27	0.27	0.5431	0.5488
45.5000	0.5744	0.27	0.27	0.5713	0.5775
47.7500	0.6028	0.28	0.28	0.5995	0.6061
50.0000	0.6313	0.28	0.28	0.6277	0.6348

Results:

NO	ABSORBANCE A	CONCENTRATION X	CONFIDENCE INTERVAL (95%) XUKI	XLKI
1.	0.0857	6.8224	6.3765	7.2648
2.	0.4460	35.3388	34.9247	35.7547
3.	0.4633	36.7106	36.2934	37.1297
4.	0.4717	37.3701	36.9514	37.7908

$$X \pm \frac{XUKI - XLKI}{2} \qquad \Delta X_{rel\%} = \frac{\frac{XUKI - XLKI}{2}}{X} 100$$

— *mean standard deviation of* x (not a true statistic data)

$$S_x = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$$

Remark: for the calculation of S_x one was used from n data, thus we have $n-1$ independent data.

The following data are given for the *characterization of the calibration line*:

at 20 equidistant points of the region calibrated

— the given concentration (x)

— the analytical signal belonging to given concentration, estimation of absorbance (A)

— *relative percentage values of the standard deviation* of the calibration line:

— in absorbance $\mathrm{ARTS\%} = \frac{S_Y(x)}{Y_{(x)}} 100\%$

— in concentration $\mathrm{XRTS\%} = \frac{S_Y(x)}{b \cdot x} 100\%$

If the hyperbola is adequately narrow, it can be substituted for parallel lines. ARTS% and XRTS% mean the distance of the parallels at the

$$S_{y(x)} = \sqrt{s_{\mathrm{res}}^2 \left(\frac{1}{n} + \frac{(x-\bar{x})^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \right)}$$

20 points selected, read at the absorbance and concentration axis, respectively, and given in relative %.

— Points of the hyperbolas forming the 95% *confidence intervals* of the calibration line:

ALCI: lower confidence interval of absorbance,

AUCI: upper confidence interval of absorbance.

$$Y_{\mathrm{conf.\,interv.}} = \bar{y} + b(x-\bar{x}) \pm t_{n-2} S_y$$

where t is the probability variable of Student's distribution belonging to the number of measurements (to $n-2$ degrees of freedom) and to the desired probability, which can be locked up in a table.

For the use of the calibration line tables contain also the following data:

— *serial numbers* of the samples

— *mean absorbance values* of parallel measurements

— *estimated concentration values* obtained with the calibration line

— *95% confidence intervals of concentration* obtained with the calibration line:

$$X_{\text{conf}}, \text{ i.e. XLCI: } X \text{ lower confidence interval}$$
$$\text{XUCI: } X \text{ upper confidence interval}$$

$$X_{\text{conf}}\begin{pmatrix}\text{XLCI}\\ \text{and}\\ \text{XUCI}\end{pmatrix} = \bar{x} + \frac{\bar{Y}-\bar{y}}{(1-\alpha^2)b} \pm \frac{\alpha}{(1-\alpha^2)b}\sqrt{\left(\frac{1}{m}+\frac{1}{n}\right)b^2(1-\alpha^2)\sum_{i=1}^{n}(x_i-\bar{x})^2+(\bar{Y}+\bar{y})^2}$$

$$\alpha^2 = \frac{t^2_{n-2}S^2_{\text{res}}}{b^2\sum_{i=1}^{n}(x_i-\bar{x})^2}$$

where: $\bar{Y}$ = mean of measurements relative to the unknown sample
m = number of measurements relative to the unknown sample
$\bar{y}$ = mean of y values obtained in the calibration measurements.

For the characterization of the analytical methods to be described the following of the above data are given:

— equation of the calibration line
— residual standard deviation (in absorbance)
— concentration values estimated for the samples analyzed, and 95% confidence interval of the concentrations estimated, which is characterized by half of the difference between the lower and upper confidence intervals given by the computer, and is written with $\pm$ sign after the estimated concentration in the following way:

$$X \pm \frac{\text{XUCI}-\text{XLCI}}{2}$$

— relative error of concentration measurement.

Experimental technique

IR spectra were taken with a Zeiss UR 10 infrared spectrophotometer, using solution and potassium bromide tablet techniques.

UV spectra were recorded with a Specord UV-VIS spectrophotometer.

Analytical procedures

Determination of chlorophacinone rodenticides

Determination of chlorophacinone in the presence of Sudan Red 7B in paraffin oil solution

Chlorophacinone is a strong poison with anticoagulating action, haemolyzing red blood cells. Marked with a colouring substance (Sudan Red 7B), it is used in paraffin solution for the killing of rodents.

The chemical structure of the active ingredient to be determined and of the accompanying (colouring) component are shown in Fig. 1.

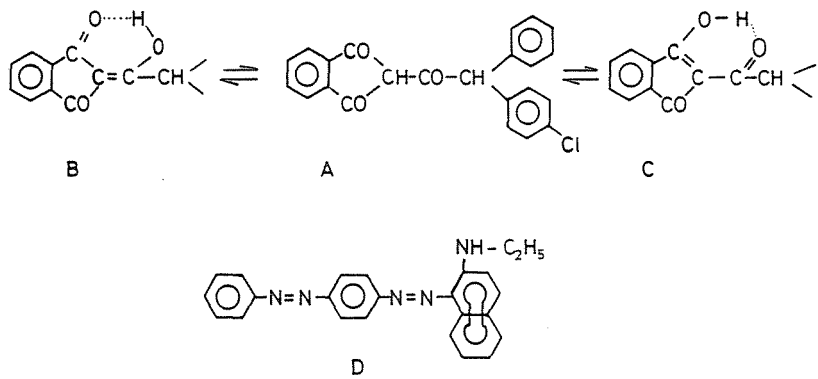


Fig. 1. The chemical structures of the components
(– The active component to be determined: chlorophacinone,
(B: enol form, A: keto form, C: enol form)
— The accompanying component: Sudan Red; D
— The solvent: Paraffin oil)

Chlorophacinone is present in the enol form. For its determination in rodenticides the C=0 stretching vibration at 1705 cm^{-1} is suitable (Fig. 2).

Expected concentration is 0.2–0.3 g chlorophacinone/100 cm^3 paraffin oil. Using the components of the samples to be analyzed and the solvent, the calibration series was prepared in the following way:

	1	2	3	4	5
Chlorophacinone (g)	0.125	0.166	0.250	0.330	0.500
Sudan Red (g)	0.100	0.133	0.200	0.266	0.400
Paraffin oil (cm^3)	100.00	100.00	100.00	100.00	100.00

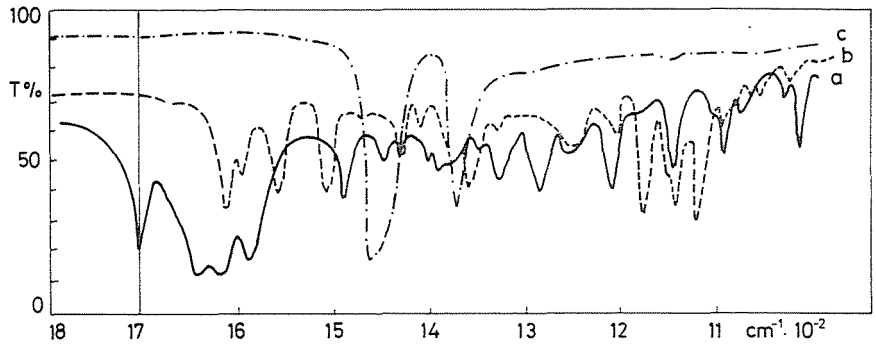


Fig. 2. The IR spectra and the determination of the analytical spot
(Chlorophacinone (a), Sudan Red (b), Paraffin oil (c))
⁎: Analytical spot

The spectra were recorded in cuvettes with NaCl window of 1.04 mm, using paraffin oil as reference. The spectrograms of the samples to be analyzed were taken under identical conditions as those of the calibration series.

Main parameters of the method developed:

Equation of the calibration line	Residual standard deviation (Absorbance)	Some estimated chlorophacinone concentrations [g/100 cm^3] ± the 95% confidence interval, relative error
$A = 0.0437 + 1.038\,X$	0.005	0.243 ± 0.005, 2% 0.261 ± 0.006, 2% 0.235 ± 0.006, 3%

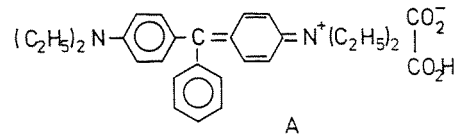
Determination of chlorophacinone in the presence of Brillian Green, talc and quartz

Rodenticides containing chlorophacinone as active ingredient are used also in the solid form. Talc and quartz are used as carrier substances, Brilliant Graen as marking dye.

The chemical structure of the component contained, and the selection of the analytical spot are shown in Figs 3 and 4.

Chlorophacinone concentration can be estimated also in this case on the basis of the absorbance of the C=0 stretching vibration band, appearing at 1705 cm^{-1}. (The band at 1730 cm^{-1} of Brilliant Green, present in a quantity of 0.5–3%, does not interfere with the determination.)

A calibration series fitting the expected composition of the samples to be analyzed (35–40% chlorophacinone content) was prepared in the following



A

SiO_2 and $Mg_3Si_4O_{10}(OH)_2$

Fig. 3. The chemical structures of the components
(— The active component to be determined: Chlorophacinone in enol form (Fig. 1.)
— The accompanying component: Brillant Green; A
— carrier substance: SiO_2 and talcum) B

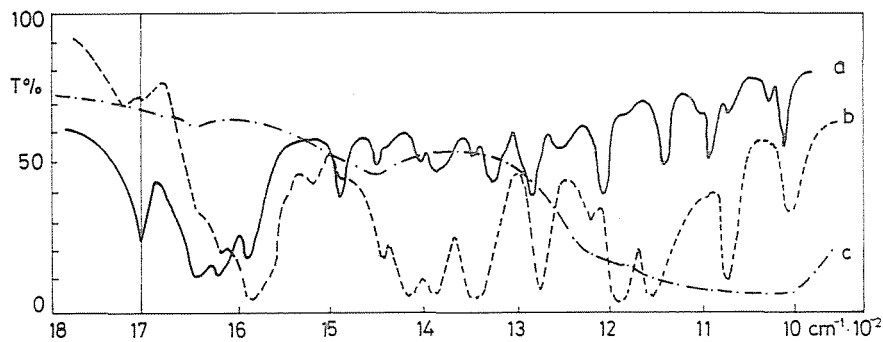


Fig. 4. The IR spectra and the determination of the analytical spot (Chlorophacinone (a), Brillant Green (b), Carrier Substance (c))
*: Analytical spot

way: stock mixtures of 5 mg/l g KBr concentration were prepared from the components, and these were compounded.

Component	Quantities weighed in from the stock mixture (g)					
Chlorophacinone	0.050	0.100	0.200	0.300	0.400	0.500
Brilliant Green	0.020	0.020	0.020	0.020	0.020	0.020
Carrier	0.930	0.880	0.780	0.680	0.580	0.480

After homogenization and tablet compressing, details of IR spectra were recorded for the members of the calibration series in the interval of the analytical spot. Under identical conditions, the spectra of the samples to be analyzed were taken on tablets of a composition of 5 mg/1g KBr sample.

Main parameters of the process elaborated:

Equation of the calibration line	Residual standard deviation (absorbance)	Some estimated chlorophacinone concentrations ±95% confidence interval, relative error
$A = 0.001 + 0.013\ X$	0.004	35.3 ± 0.4%, 1% 36.7 ± 0.4%, 1% 37.3 ± 0.4%, 1%

Determination of the active ingredient in insecticides

Determination of the active ingredient of code name GM in the presence of an antioxidant

The active ingredient GM is the luring substance of Grapholitha molesta (fruit fly), the pest of apple orchards. It is spread with an antioxidant on rubber capsules and placed into boxes caoted with adhesive. From the number of insects captured, the most efficient spraying time can be determined. Our method of determination serves for the measurement of the active ingredient, dissolved from the capsule with carbon tetrachloride.

The chemical structure of the components contained, details of the IR spectrogram and selection of the analytical spot are shown in Fig. 5.

The C=0 stretching vibration of the ester group of the active ingredient, appearing at 1740 cm^{-1}, forms the basis of the quantitative measurement. Taking into consideration the expected GM concentration, solutions in carbon tetrachloride were prepared in the interval 0.1–10 mg/cm^3, to which the antioxidant was added in the same ratio as that in the samples.

For the analysis of the samples, the active ingredient and the antioxidant were washed off with carbon tetrachloride from a few capsules. Excess solvent was evaporated, the flask was filled up to the mark, and under identical conditions as in calibration, the spectra of the solutions obtained were recorded in 1 mm cuvettes with NaCl window. From absorbance values calculated at 1740 cm^{-1} the concentration of the active ingredient was estimated with the aid of the calibration line.

Main parameters of the method elaborated:

Equation of the calibration line	Residual standard deviation	Some estimated concentrations ±95% confidence interval, relative error
$A = 0.054 + 0.147\ K$	0.006	1.1 ± 0.10 mg/cm^3, 10% 1.5 ± 0.08 mg/cm^3, 5% 1.7 ± 0.09 mg/cm^3, 5%

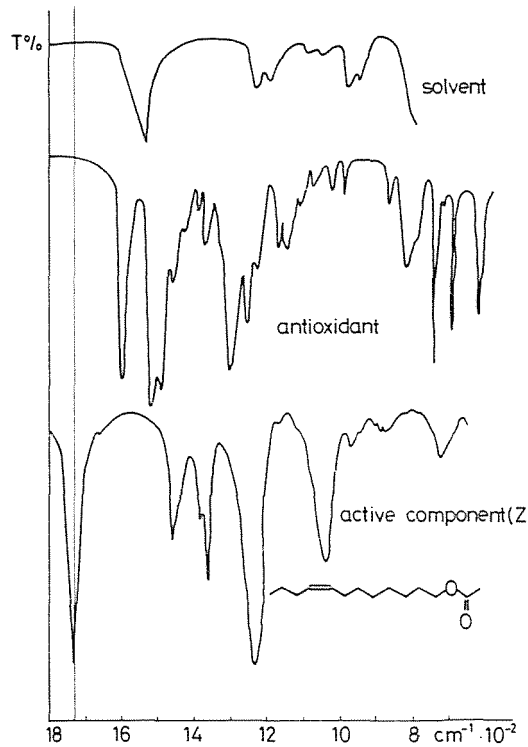


Fig. 5. The chemical structure and the determination of the analytical spot
(CCl_4: Solvent, Antioxidant; The active component: GM)
[8-dodecene — 1-ol-acethate (Z)]
*: Analytical spot

Ten capsules were used for each analysis, and after dissolution the solution was evaporated to 10 cm^3.

Carbon tetrachloride was used as reference, obtained by treating in an identical way an identical number of empty capsules as used for sample preparation.

Determination of the active ingredient UP in the presence of an antioxidant

UP active ingredient is the sexual pheromon (luring substance) of Laspsyresia pomonella (apple fly). Similarly as GM, it is sold spread on rubber capsules.

Our method of determination measured the active ingredient in the sample dissolved with ethyl alcohol from the capsule. The active ingredient UP is considerably more volatile than GM, thus removal of excess solvent after

dissolution caused losses. Therefore, a method of determination by UV spectrophotometry was elaborated for more dilute solutions.

The structure and UV spectrum of the active ingredient and the UV spectrum of the antioxidant are shown in Fig. 6.

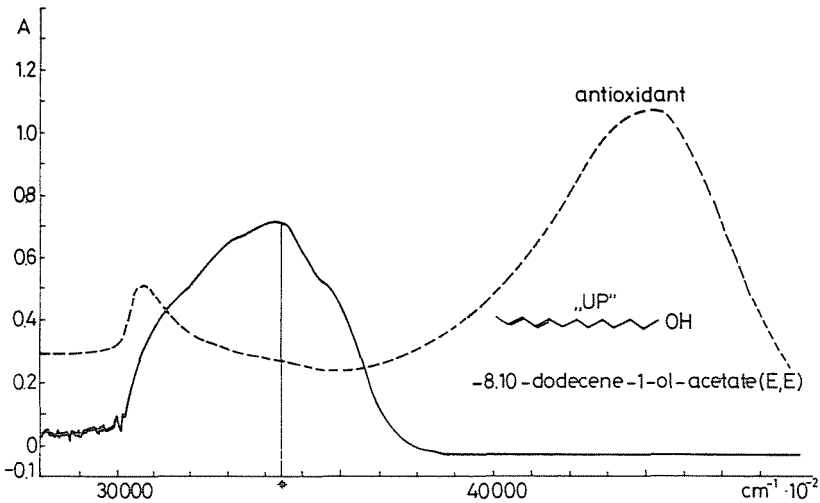


Fig. 6. The chemical structure of the active component and the determination of the analytical spot
(UP: The active component, antioxidant)
⁎: Analytical spot

The band of the active substance appearing at 34 385 cm^{-1} is suitable for quantitative determination without previous separation, because at this range neither the accompanying antioxidant nor the solvent reveal absorption. A series of solutions in ethyl alcohol were prepared for calibration, under consideration of the composition of the sample with respect to concentration ratio and interval, in which the concentration of the active ingredient varied between 5 and 50 $\mu g/cm^3$. Knowing the concentration dependence of absorbance at this wavelength, the active ingredient of the sample, dissolved from the capsules with ethyl alcohol, was measured. The solution obtained by treating with alcohol capsules without active ingredient was used as reference.

The main parameters of the process elaborated are the following:

Equation of the calibration line	Residual standard deviation	Some estimated concentrations ±95% confidence interval and relative error
$A = 0.014 + 0.025\,X$	0.009	28.0±0.9 μg/10 cm^3, 3% 27.2±0·9 μg/10 cm^3, 3% 30.1±0.9 μg/10 cm^3, 3%

Determination of carbofurane in soil disinfectants

In soil disinfectants containing carbofurane several accompanying substance are used besides the active ingredient. A method was developed for the analysis of the following four kinds of the preparations on the market:

1. Carbofurane (C), dimethyl formamide (DMF) and fugrane (F)
2. Carbofurane (C), dimethyl formamide (DMF) and pearl granule (PG)
3. Carbofurane (C) and fugrane (F)
4. Carbofurane (C), dimethyl formamide (DMF), perlite (P) and fugrane (F).

The chemical structure of the components and details of their IR spectra are shown in Figs 7/a–d.

For the quantitative determination of carbofurane without preliminary separation the dependence on carbofurane concentration of the absorbance of the C=0 stretching vibration band appearing at 1730 cm^{-1} is suitable in the case of all the four kinds of soil disinfectants.

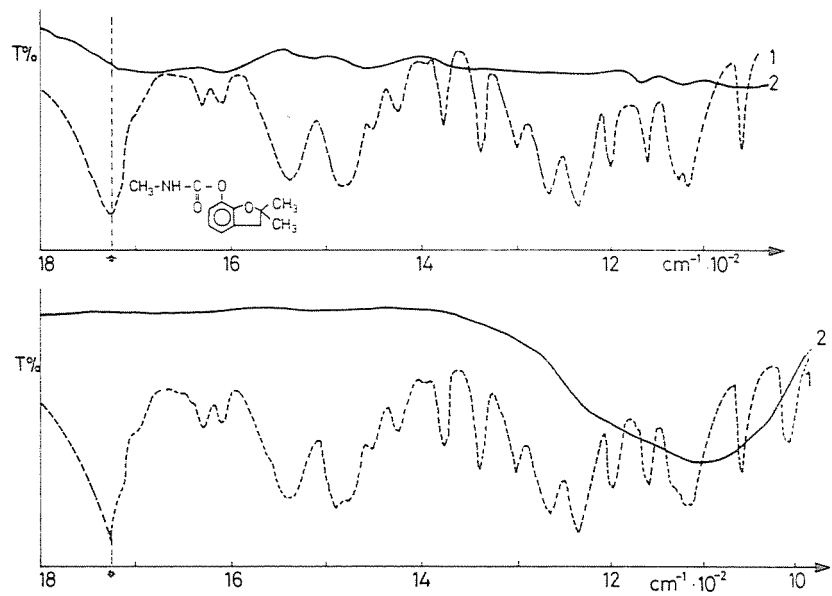


Fig. 7.a. The chemical structure of the active component and the determination of analytical spot
— carbofurane (1)
— fugrane + dimethylformamide (DMF) 17 + 2 (2)
*: Analytical spot

Fig. 7.b. The chemical structure of the active component and the determination of analytical spot
— carbofurane (1)
— DMF + pearlgranule (2 + 17) (2)
*: Analytical spot

Calibration was performed by weighing in calibration series suiting the composition of the samples to be analyzed, and by the evaluation of their IR spectra.

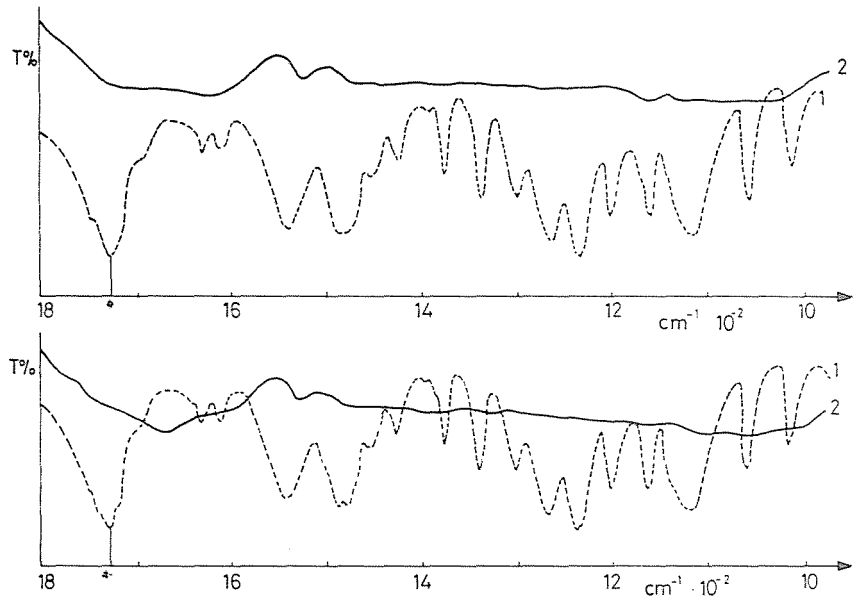


Fig. 7.c. The chemical structure of the active component and the determination of analytical spot
— carbofurane (1)
— fugrane (2)
*: Analytical spot

Fig. 7.d. The chemical structure of the active component and the determination of analytical spot
— carbofurane (1)
— DMF + perlite + fugrane (2)
*:Analytical spot

In the recording of the spectra a tablet was used as reference substance, which contained with the exception of the active ingredient all the components in the same concentrations as in the samples to be analyzed.

From the samples to be analyzed potassium bromide tablets were prepared in the same way as used for the preparation of the calibration series, and carbofurane concentrations were estimated from absorbance values at 1730 cm^{-1} with the aid of the calibration lines.

Main parameters of our process developed are contained in the following table:

Components present besides carbofurane	Equation of the calibration line	Residual standard deviation (absorbance)	The most frequent carbofurane concentrations in the samples and the 95% confidence interval, relative error
Dimethyl formamide	$y=0.044\pm0.031x$	0.018	15.0 ± 0.5 mg/cm^3, 3%
Dimethyl formamide + fugrane 2+17	$y=0.018+0.023x$	0.009	$10.0\pm0.5\%$, 5%
Dimethyl formamide + pearl granule	$y=0.023+0.025x$	0.007	$10.0\pm0.3\%$, 3%
Fugrane	$y=0.019+0.023x$	0.006	$10.0\pm0.3\%$, 3%
Dimethyl formamide + perlite + fugrane	$y=0.011+0.024x$	0.010	$10.0\pm0.5\%$, 5%

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