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## ANALYSIS

# DETERMINATION OF ACTIVE SUBSTANCES IN BINARY MIXTURE ANTIPARASITIC VETERINARY FORMULATIONS BY HPLC

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**Abstract:** The purpose of the study was to develop a simple, versatile HPLC method for the identification and quantification of praziquantel and ivermectin (in Equimax) or praziquantel and abamectin (in Abamitel Plus). A satisfactory separation was obtained using the Supelcosil LC-ABZ+ column in gradient system with a mobile phase A: acetonitrile / water in 40:60 ratio and phase B: acetonitrile. The UV detection was set at 245 nm. The correlation coefficient values (≥ 0,998) for all active substances confirmed that the calibration curves (peak area *vs*. concentration) are linear The results of the quantification and the statistical evaluation confirmed that the method is accurate and precise. It can also be applied to confirm the identity of benzyl alcohol, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate in Abamitel Plus formulation.

**Keywords**: praziquantel, ivermectin, abamectin, HPLC method

Parasitosis is a common infection in animals. The horses, due to domestication, grouping and reduced breeding area are particularly exposed to parasitic diseases. The de-worming procedure is carried out twice a year, before and after the grazing season.

The most effective multicomponent drugs are composed of active substances with different action modes. They include oral pastes: Abamitel Plus and Equimax. The drugs contain abamectin or ivermectin in combination with praziquantel. Both ivermectin and abamectin are part of the avermectin group.

They increase γ-aminobutyric acid (GABA) release in neurons of central and peripheral nervous systems, causing an irreversible nerve palsy and death of the parasites.

Praziquantel is a pyrazine derivative of isocholine. It is effective against tapeworms, by increasing calcium ion flow through the tapeworm integument, increasing the calcium level in the muscle cells, and causing palsy and spasms. Figure 1 shows structural and molecular formulas of analyzed compounds. The analyzed drugs are pastes containing several excipients, e.g., benzyl alcohol, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate.

The purpose of the study was to develop a simple, versatile HPLC method for identification and quantification of praziquantel, ivermectin or abamectin in binary pharmaceutical formulations: Abamitel Plus and Equimax

An available literature contains only sparse studies related to the determination of abamectin, ivermectin or praziquantel in drugs. The analysis was carried out by colorimetric (1), voltamperometric (2), spectrophotometric (3) and chromatographic (4–8) methods. In a single study, a quantification of abamectin with praziquantel in veterinary oral paste by HPLC in isocratic system was described (8). Other publications deal with the determination of a single active substance or its metabolites in a biological material – fruits and food (9–12), milk (13–15), meat and animal tissues (16–20), plasma (21–28), and environment (29–31).

## EXPERIMENTAL

### Materials

The following materials were used: Drugs: Abamitel Plus paste containing praziquantel 50 mg/mL + abamectin 4 mg/mL, produced by KRKA; Equimax paste containing praziquantel 150 mg/g +

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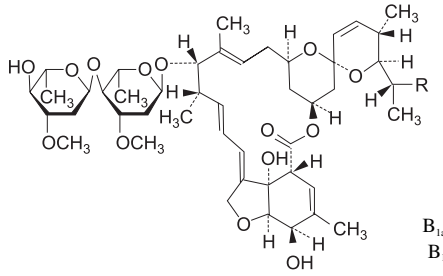
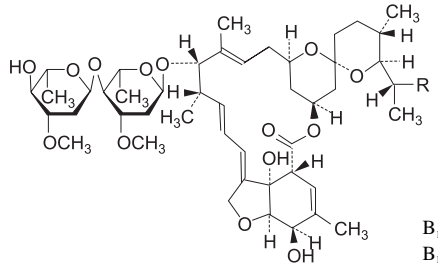


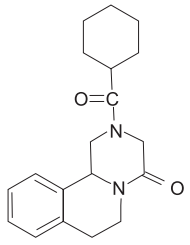
$B_{1a}$ $C_{48}H_{72}O_{14}$
$B_{1b}$ $C_{47}H_{70}O_{14}$

Abamectin
Component $B_{1a}$ R = $C_2H_5$: 5-O-demethylavermectin $A_{1a}$
Component $B_{1b}$ R = $CH_3$: 5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl) avermectin $A_{1a}$



$B_{1a}$ $C_{48}H_{74}O_{14}$
$B_{1b}$ $C_{47}H_{72}O_{14}$

Ivermectin
Component $B_{1a}$ R = $C_2H_5$: 5-O-demethyl-22,23-dihydroavermectin $A_{1a}$
Component $B_{1b}$ R = $CH_3$: 5-O-demethyl-25-de(1-methylpropyl)- 22,23-dihydro-25-(1-methylethyl) avermectin $A_{1a}$



$C_{19}H_{24}N_2O$

Praziquantel

Figure 1. Structural and total formulae of the studied compounds

ivermectin 12 mg/g produced by VirbacAnimal Health. Standards: Praziquantel from Bayer AG, Abamectin from KRKA, and Ivermectin CRS Ph.Eur.

**Reagents and apparatus:**

Acetonitrile and methanol were of HPLC grade. Shimadzu LC system with PC interface, UV-VIS SPD-10AVVP detector, LC-10ATVP pumps, DGU-14A degasser, SCL-10AVP controller, and SIL-10ADVP autosampler were used.

**Standard solutions**

Methanolic solutions of standards at a concentration of: 1.5 mg/mL for praziquantel and 0.12 mg/mL for abamectin and ivermectin.

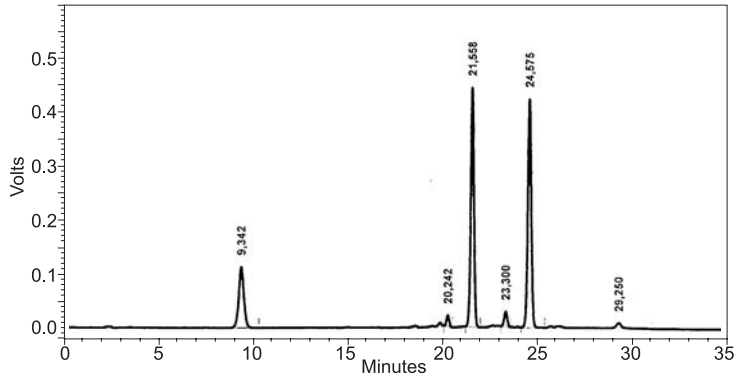



Figure 2. Chromatogram of a mixture of active substances. Retention times: praziquantel 9.34 min; abamectin $B_{1b}$ 20.24 min; abamectin $B_{1a}$ 21.56 min; ivermectin $B_{1b}$ 23.30 min; ivermectin $B_{1a}$ 24.58 min; peak at 29.2 min retention time is the mobile phase peak. Resolutions: praziquantel – abamectin $B_{1b}$ 28.19; abamectin $B_{1b}$ – abamectin $B_{1a}$ 4.44; abamectin $B_{1a}$ – ivermectin B1b 5.61; ivermectin B1b – ivermectin B1a 3.94

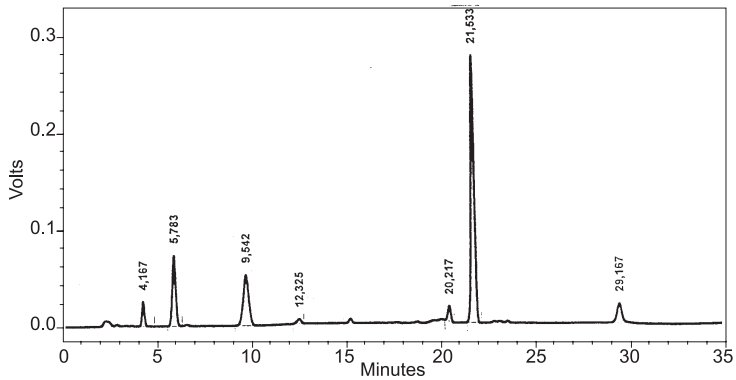



Figure 3. Chromatogram of the Abamitel Plus paste. Retention times of the active substances and excipients: benzyl alcohol 4.17; methyl *p*-hydroxybenzoate 5.78 min; praziquantel 9.54 min; propyl *p*-hydroxybenzoate 13.32 min; abamectin $B_{1b}$ 20.22 min; abamectin $B_{1a}$ 21.53 min; peak at 29,2 min retention time is the mobile phase peak

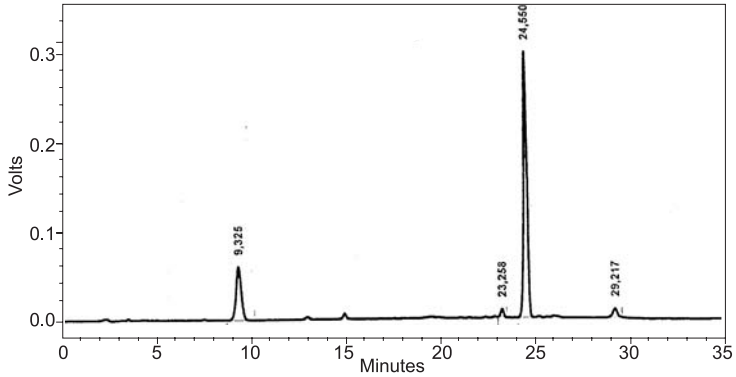



Figure 4. Chromatogram of the Equimax paste. Retention times: praziquantel 9.32 min; ivermectin $B_{1b}$ 23.26 min; ivermectin $B_{1a}$ 24.55 min; peak at 29.2 min retention time is the mobile phase peak

Table 1. Calibration curve parameters, correlation coefficients, detection limits (LOD) and quantification limits (LOQ) of the analyzed substances.

| Substance determined | Calibration curve parameters y = ax + b | LOD [mg/mL] | LOQ [mg/mL] |
|---|---|---|---|
| Praziquantel | a ± Δa = 673515 ± 37155; $S_a$ = 11675<br>b ± Δb = 181905 ± 71639; $S_b$ = 22511<br>$S_y$ = 21352; r = 0.9995 | 0,105 | 0,317 |
| Abamectin $B_{1a}$ | a ± Δa = 31498533 ± 1141406; $S_a$ = 411102<br>b ± Δb = 33141 ± 120371; $S_b$ = 43354<br>$S_y$ = 45832; r = 0.9996 | 0,005 | 0,014 |
| Abamectin $B_{1b}$ | a ± Δa = 30139132 ± 1054081; $S_a$ = 379651<br>b ± Δb = -2222 ± -9694; $S_b$ = 1891<br>$S_y$ = 2000; r = 0.9997 | 0,0002 | 0,0007 |
| Ivermectin $B_{1a}$ | a ± Δa = 34622346 ± 1026518; $S_a$ = 369723<br>b ± Δb = 44736 ± 101500; $S_b$ = 36558<br>$S_y$ = 38128; r = 0.9998 | 0,004 | 0,011 |
| Ivermectin $B_{1b}$ | a ± Δa = 29722855 ± 3864750; $S_a$ = 1214395<br>b ± Δb = 10406 ± 29308; $S_b$ = 9209<br>$S_y$ = 6659; r = 0.9975 | 0,0007 | 0,0002 |

a, b – regression coefficients; Sa, Sb – standard deviation of regression coefficients; $S_y$ – standard error of the estimate; r – correlation coefficients

Table 2. Results of recovery in Abamitel Plus paste.

| Praziquantel | | | Abamectin | | |
|---|---|---|---|---|---|
| Amount added [mg] | Amount found [mg] | Recovery [%] | Amount added [mg] | Amount found [mg] | Recovery [%] |
| 106.46 | 104.74 | 98.38 | 9.06 | 8.92 | 98.45 |
| 97.63 | 97.99 | 100.37 | 8.34 | 8.26 | 99.08 |
| 100.96 | 101.20 | 100.24 | 8.60 | 8.68 | 100.93 |
| 113.14 | 112.87 | 99.76 | 9.46 | 9.54 | 100.84 |
| 118.26 | 117.62 | 99.46 | 9.89 | 9.86 | 99.70 |
| 111.55 | 112.45 | 100.81 | 9.35 | 9.36 | 100.16 |
| Mean: 99.84 ± 0.68<br>SD 0.86<br>%RSD 0.86 | | | Mean 99.86 ± 0.78<br>SD 0.98<br>%RSD 0.98 | | |

Table 3. Results of recovery in Equimax paste.

| Praziquantel | | | Abamectin | | |
|---|---|---|---|---|---|
| Amount added [mg] | Amount found [mg] | Recovery [%] | Amount added [mg] | Amount found [mg] | Recovery [%] |
| 160.22 | 161.24 | 100.64 | 12.69 | 12.61 | 99.34 |
| 159.34 | 160.89 | 100.97 | 12.55 | 12.71 | 101.31 |
| 161.39 | 163.80 | 101.49 | 12.61 | 12.53 | 99.37 |
| 176.80 | 178.95 | 101.22 | 13.79 | 13.71 | 99.38 |
| 175.29 | 177.88 | 101.48 | 13.63 | 13.44 | 98.61 |
| 174.98 | 178.18 | 101.83 | 13.66 | 13.46 | 98.55 |
| Mean: 101.27 ± 0.34%<br>SD 0.42<br>%RSD 0.42 | | | Mean 99.43 ± 0.80%<br>SD 1.00<br>%RSD 1.00 | | |

Table 4. Results of the active substanes assay in Abamitel Plus and Equimax.

| Drug product | Active substance | Declared amount of active substance | Found amount of active substance | | | |
|---|---|---|---|---|---|---|
| | | | n | Mean | SD | %RSD |
| Abamitel Plus | Praziquantel | 50.0 mg/mL | 6 | 49.7 ± 0.37 mg/mL | 0.46 | 0.93 |
| | Abamectin | 4.0 mg/mL | 6 | 4.2 ± 0.03 mg/mL | 0.03 | 0.78 |
| Equimax | Praziquantel | 150.0 mg/g | 6 | 144.8 ± 1.07 mg/g | 1.33 | 0.92 |
| | Ivermectin | 12.0 mg/g | 6 | 11.5 ± 0.08 mg/g | 0.10 | 0.91 |

**Test sample solutions**

1.5 g Abamitel Plus paste was transferred to 50 mL volumetric flask (corresponding to 1.5 mg/mL praziquantel and 0.12 mg/mL abamectin).

1 g Equimax paste was transferred to 100 mL volumetric flask (corresponding to 1.5 mg/mL praziquantel and 0.12 mg/mL ivermectin).

Both samples were diluted with methanol, shaken by mechanical shaker, diluted to volume with the same solvent and filtered.

## RESULTS AND DISCUSSION

Firstly, an optimal system for identification and separation of analyzed compounds and excipients was searched. Several chromatographic systems were verified (columns and mobile phases). The following system was selected: Supelcosil LC-ABZ+ 5 mm, 250 × 4.6 mm column; mobile phase A containing a mixture of acetonitrile and water (40:60, v/v), mobile phase B containing acetonitrile used in the gradient system (time, min/%B): 0–5/0; 5–20/0 → 80; 20–25/80; 25–30/80 → 0; 30–35/0.

A mobile phase flow rate was 1.2 mL/min. Detection at 245 nm (UV-VIS detector) was applied. Figure 2 shows the chromatogram for a standard mixture. Both ivermectin and abamectin are composed of two compounds ($B_{1a}$ and $B_{1b}$), and generate two peaks each. The values of retention times and resolution between the neighbor peaks are also shown in the Figure caption.

**Specificity**

To verify the specificity of the method, the solvent, the solutions of standard active substances, of excipients and of praziquantel impurities (A, B and C acc. to Ph. Eur.) were injected into the column. The peaks of the solvent, impurities and excipients do not interfere with the peaks of the active substances.

**Linearity**

A linearity of the relation between peak surface areas and concentrations of each compound was verified for the following concentration ranges: praziquantel 0.7 mg/mL to 3,0 mg/mL; abamectin (component $B_{1a}$) 0.03 mg/mL to 0.15 mg/mL; abamectin (component $B_{1b}$) 0.001 mg/mL to 0.01 mg/mL; ivermectin (component $B_{1a}$) 0.03 mg/mL to 0.15 mg/mL; ivermectin (component $B_{1b}$) 0.004 mg/mL to 0.010 mg/mL. Table 1 shows calibration curve parameters with correlation coefficients.

**Limit of detection and limit of quantification**

A limit of detection (LOD) and a limit of quantification (LOQ) were established based on the calibration curve parameters, according to the formula: LOD = 3.3×Sy/a and LOQ = 10×Sy/a, where Sy = standard error of estimate and a = slope of the regression line. Table 1 shows the results.

**Precision**

Standard solutions were injected to the column six times and the results were subjected to statistical evaluation. The RSD of the peak areas ranged from 0.2% (ivermectin $B_{1a}$) to 0.4% (abamectin $B_{1b}$).

**Accuracy**

Know amount of abamectin, ivermectin and praziquantel working standards were added to the drug products to obtain 110% and 120% of the declared quantity of active substances. The samples were diluted with methanol for content detemination. Ten µL samples of prepared solution were injected into the column. Tables 2 and 3 show the results.

**Content determination**

Ten µL samples of the prepared standard and sample solutions were injected into the column.

Figures 3 and 4 show the sample chromatograms. Abamectin and ivermectin content was calculated as a sum of two peaks corresponding to the $B_{1a}$ and $B_{1b}$ components. Table 4 shows the results.

## CONCLUSIONS

The described HPLC method enables identification and quantification of praziquantel, abamectin and ivermectin in veterinary oral pastes. The method can also be used for determination of several excipients (benzyl alcohol, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate) and impurites. The correlation coefficients value (exceeding 0.998) for all active substances confirmed that calibration curves are linear. The results of injections of standard solutions into the column (RSD from 0.2% to 0.4%) demonstrate that the method is precise. Recovery results are in the range 98.5–101.3%, which prove the suitability and accuracy of the proposed method. Assay results of active substances are in good agreement with the declared content. The proposed HPLC method could be used for routine analysis.

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