# A New Approach to the Extraction of Substances from a Spot on a Chromatographic Plate

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## INTRODUCTION

Combining thin-layer chromatography with modern techniques of instrumental analysis has recently become common in chemical analysis. One of many examples of this combination involves extracting a substance from a spot on a chromatography plate and transferring the resulting solution to a mass spectrometer. Several devices on the market can be used for this task; however, they suffer from the drawback of a relatively high price. Our presentation shows a simplified approach to extracting substances from a chromatographic plate. Based on the example of the extraction of selected coccidiostats (maduramicin, narasin, salinomycin, monensin, lasalocid and nigericin - antibiotics/chemotherapeutics allowed for use in animals, mainly poultry, in the prevention or treatment of a disease called coccidiosis, caused by Eimeria protozoa), we present some validation parameters for the analysis of test samples.

## AIM

The research aimed to analyze solutions prepared using a prototype extractor with the LC-MS technique. The goal was to determine whether there is a linear dependence of the peak area of the examined substance on its concentration in the obtained solutions. Specifically, we looked at the ratio of the peak area of the substance to the peak area of the internal standard, maintaining a constant concentration of the latter. The study focused on five selected coccidiostats: maduramycin, narasin, salinomycin, monensin, and lasalocid, with nigericin used as the internal standard.

## EXPERIMENTAL

**Preparation of solutions.** The concentration of the coccidiostats in the methanol samples was 0.1, 0.4, 0.9, 2.0, 4.0, 8.0, and 12.0 mg/L, respectively. The concentration of the standard was 0.25 mg/mL. In the case of solutions of substances added to poultry feed, 2.5 g of feed was weighed into a 50 ml polypropylene centrifuge tube. Then, working solutions prepared from the stock solutions were added so that the final concentration of coccidiostats in the sample was 0.1, 0.4, 0.9, 2.0, 4.0, 8.0, and 12.0 mg/kg, respectively. The concentration of the standard solution was 0.25 mg/kg. The sample was then shaken, 10 ml of acetonitrile was added, and the sample was again vigorously mixed.

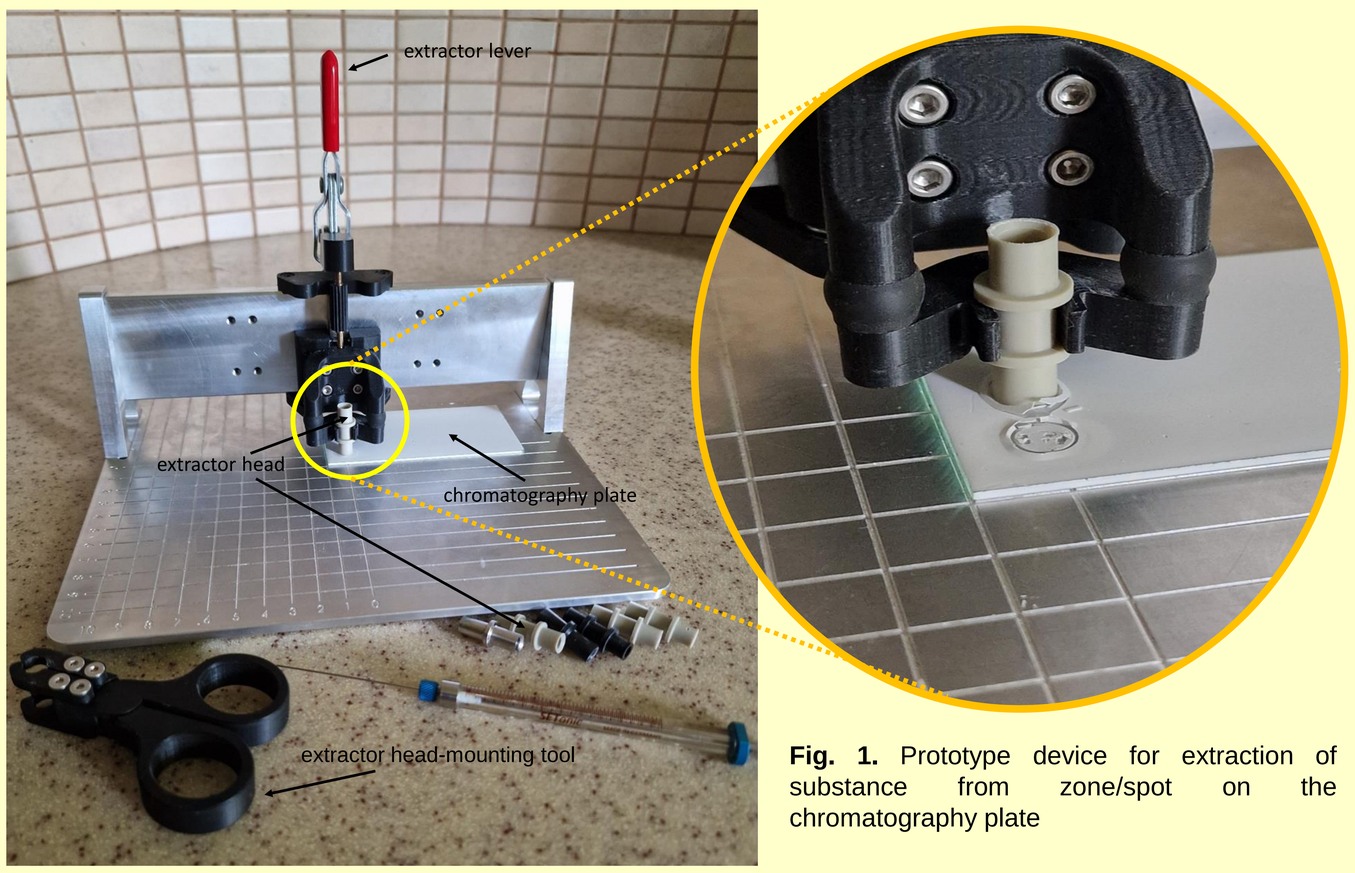


**Fig. 1.** Prototype device for extraction of substance from zone/spot on the chromatography plate

**EXTRACTION PROCESS**: The extraction process was conducted in a solid-liquid system (an adsorbent layer of the chromatographic plate - methanol) with the prototype extractor (Fig. 1) Solutions of the studied coccidiostats, each with a volume of 5 µL, were applied onto an adsorbent layer of the chromatographic plate with silica gel (HPTLC Silica gel 60 F254) using an automatic pipette and left to dry (Fig. 2A). Subsequently, the extractor head was pressed against the adsorbent layer of the chromatographic plate using the extractor lever, ensuring the extractor head covered the entire zone of the substances (Fig. 2B). For the extraction of each zone of substances, a single portion of methanol with a volume of 50 µL was used (single-stage extraction). This portion of methanol was introduced using a syringe equipped with a 90° flat-tip needle (250 µL syringe) into the extractor head, covering the entire zone of substances to be extracted (Fig. 2C). The extraction process (Fig. 2D) was conducted for 5 minutes. After extraction, the solutions (extracts) were retrieved from the extractor head (Fig. 2E) using the mentioned syringe and transferred to 100 µL vials. These solutions were then filtered to remove solid particles originating from the chromatographic plate's adsorbent layer. Then, the extractor lever was released (Fig. 2F), and the extraction of the next zone of substances began.

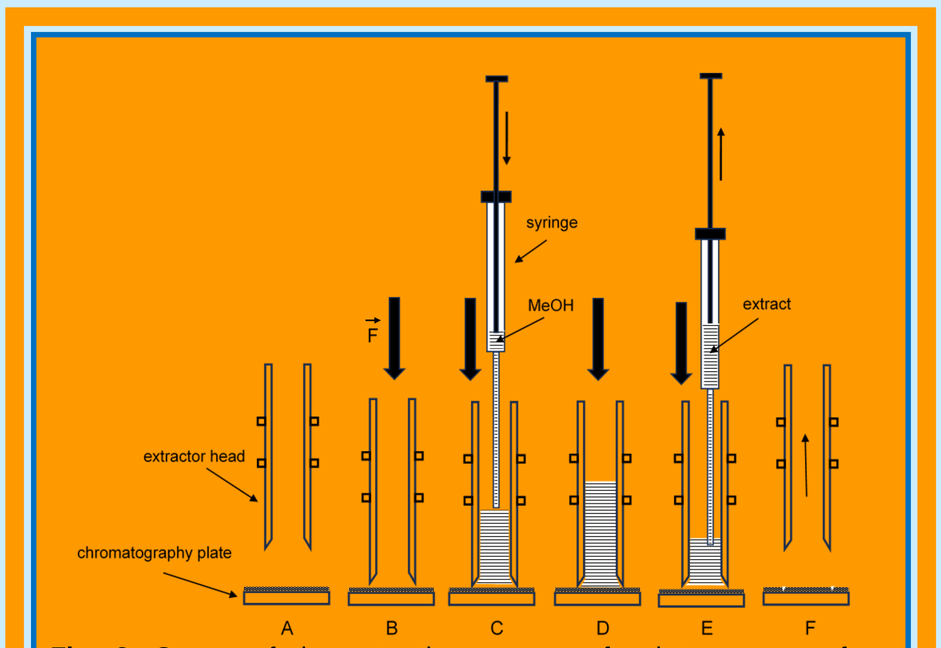


**Fig. 2.** Stages of the extraction process of substance zone from the chromatography plate using the prototype extractor.

**LC-MS analysis**: The obtained extracts were analyzed by the LC-MS technique. Chromatography: Agilent 1290 Infinity LC System; mobile phase: 95% methanol with 0,1 % formic acid, 5% water with 0,1 % formic acid; stationary phase: Zorbax Eclipse Plus C18, 4,6 x 100 mm; 3,5 µm (Agilent, USA). Mass spectrometry: Agilent 6460 Triple Quad, ESI Jet Stream (+).

## RESULTS

The studies concerning the determination of the correlation were conducted in two variants:

a) For methanol solutions of coccidiostats. These solutions were applied to the TLC plate and extracted using the prototype extractor.

b) For solutions of substances added to poultry feed with concentrations ranging from 0.1 to 12 mg/kg. These were extracted using the prototype extractor from a single zone on the chromatographic plate.

Based on the obtained results, the determination coefficient was calculated for five selected coccidiostats for two experimental variants. The determination coefficient was calculated for two samples (each containing five examined substances; the study was repeated three times). The results obtained from the experimental studies are presented in Figs. 3A and 3B.

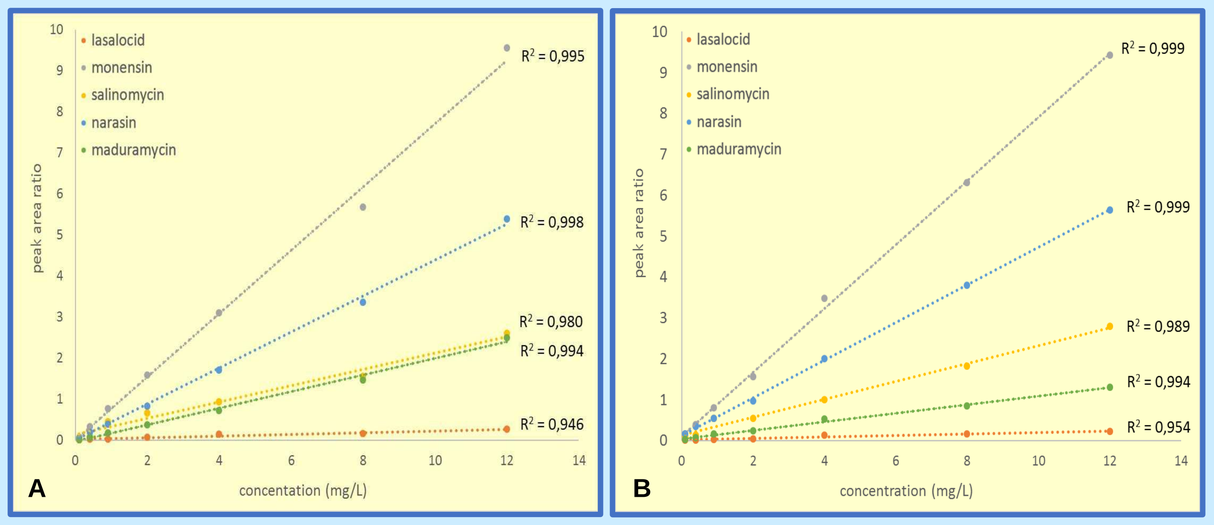


**Fig. 3.** Dependence of the ratio of peak areas of the studied coccidiostats to the peak area of the internal standard on the concentration obtained for methanol solutions (**A**) and solutions of substances added to poultry feed (**B**).

## CONCLUSIONS

The extraction device presented in this communication can be successfully used in current laboratory practice to prepare samples for further analysis using instrumental techniques.

## ACKNOWLEDGEMENTS

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