Determination of Imazalil by on-line Coupled Capillary Isotachophoresis with Capillary Zone Electrophoresis

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Abstract: A simple, rapid and reproducible capillary isotachophoretic on-line coupled with capillary zone electrophoresis (CITP-CZE) method for the determination of IMZ in food packaging extracts and its residues in apples is described. A good separation of the IMZ from other sample constituents was achieved within 15 minutes without any sample clean up. Method characteristics (linearity, accuracy, intra-assay and detection limit) were determined. Less amount of time involved, sufficient sensitivity and low running cost are the important attributes of CITP-CZE method.

Keywords: imazalil, pesticide, packaging, capillary isotachophoresis and capillary zone electrophoresis

1 Introduction

Imazalil is a systemic imidazole fungicide used to control a wide range of fungi on fruit, vegetables, and ornamentals, including powdery mildew on cucumber and black spot on roses. Imazalil is also used as a seed dressing and for post harvest treatment of citrus, banana, and other fruit to control storage decay. For example, in the Czech Republic, Imazalil is permitted for cucumber, banana and citrus treatment and its residual content in these fruits cannot exceed 0.2 mg.kg$^{-1}$, 2 mg.kg$^{-1}$ and 5 mg.kg$^{-1}$, respectively. Imazalil was also proposed as active agent in several food-packaging systems [1], [2].

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part of a research program directed at the study of similar systems of packaging in our laboratory, the aim of presented work was to develop a method for convenient determination of imazalil migration into standard food simulants as well as into real foodstuffs, i.e. packaged fruit.

![Chemical structure of imazalil](image)

**Fig. 1** Chemical structure of imazalil (1-[2-allyloxy-2-(2,4-dichloro-phenyl)-ethyl]-1H-imidazole, CAS 73790-28-0, M.wt. 297.2)

Several methods are used for the determination of IMZ residues in real samples. Chromatographic methods using different separation principle (HPLC, GC, TLC) and detection (ultraviolet, fluorimetric, electron capture, nitrogen-phosphorus, mass-spectrometry) are very common [3], [4], [5], [6]. But this methods have some drawbacks. All techniques usually need sample pre-treatment, e.g., liquid-liquid extraction, solid phase extraction or ion exchange clean up. Typical LOD of these methods varies from 0.05 to 1 µg of IMZ.g⁻¹.

An on-line capillary isotachophoresis – capillary zone electrophoresis (CITP-CZE) method for IMZ determination is presented as alternative to the above mentioned techniques.

It has been shown [7], [8] that this combination of ITP and ZE is suitable for analysis of trace ionogenic constituents present in a large excess of matrix ions. The CITP-CZE mode utilizes advantages of both methods. The ITP step enables injection of large amounts of a sample (up to several hundred microliters) and thus permits analysis of ionogenic constituents below mmol L⁻¹. The sample constituents are separated into a stack of the zones with minor constituents focused into narrow bands. Bulk ionogenic components are forced to migrate out of the separation compartment at the end of the preseparation column. The minor analytes (e.g. imazalil) concentrated and cleaned up from the bulk component in the ITP step are transferred into the analytical column as a narrow sample pulse in the ZE step. The removal of bulk component is well defined and reproducible when it is based on the signal from the conductivity detector of the preseparation column. The ZE step offers high resolution and aids in the identification of minor components using migration times. The CITP-CZE is a simple (no sample pre-treatment), quick, sufficiently sensitive and low running cost method and therefore well suited for routine analysis.
2 Experimental

2.1 Chemicals

Standard of imazalil (IMZ), and ε-aminocaproic acid (EACA) were purchased from Sigma-Aldrich, Ltd. (Prague, Czech Republic). Ammonium hydroxide, acetic acid (HAc) and methanol were obtained from Lachema (Brno, Czech Republic). Ammonium hydroxide and HAc were purified by conventional method prior to use for the electrolyte preparation. Deionised water and methanol were used for electrolyte, standard solutions, and sample preparation.

Samples of packaging with immobilised IMZ were prepared in our laboratory and the apple was obtained from local market.

2.2 Apparatus

The electrophoretic analyser used was an EA 100 (LABECO-VILLA, Ltd., Slovak Republic) with column coupling. The separation was performed in a FEP pre-separation capillary (90 mm x 0.8 mm ID), which was coupled with a FEP (fluorinated ethylene-propylene copolymer) analytical capillary (320 mm x 0.3 mm ID, length to detector was 240 mm). Zones were detected by a conductivity (preseparation capillary) and UV detector LCD2084 (ECOM, Ltd., Czech Republic) at 220 nm, respectively. The electropherograms were evaluated by PC software package supplied with the analyser.

2.3 Conditions of CITP-CZE analysis

The isotachophoretic step (preseparation capillary) of cationic analysis of IMZ was performed with leading electrolyte comprising of 5 mM-NH₄OH + 10 mM- (HAc) + 25% MeOH and a terminating electrolyte of 5 mM-HAc + 25 % MeOH. A mixture of 10 mM-HAc + 10 mM- (EACA) in 25 % MeOH serves as a background electrolyte for zone electrophoretic step (analytical capillary). The driving current applied on the preseparation capillary and analytical capillary was 150 μA and 30 μA, respectively. The samples were injected via sample valve of 35 μl fixed volume or with the help of a 10-μl Hamilton syringe. Each analysis required 15 minutes.

2.4 Calibration

The external standard method was used. Standard of Imazalil was injected (in duplicate) from the 2 mg mL⁻¹ MeOH stock solution at five-levels (5 to 100 mg mL⁻¹ 25% MeOH) using sampling valve with fixed volume (35 μl).
## 2.5 Sample treatment

Water or ethanol extracts\(^\dagger\) of packaging material with immobilized IMZ was diluted (10-times to 100-times) with water and made up with MeOH to 25 % MeOH. Oil extracts\(^\ddagger\) of packaging material\(^\ddagger\) with immobilized IMZ were extracted with 25% MeOH and diluted (if necessary) with 25 % MeOH prior to analysis. Samples of mashed apples (10 g each) were extracted with 50 mL of methanol for 20 minutes in an ultrasonic bath. Filtrate (through paper) was diluted with water to 25 % MeOH and used for CITP analysis.

## 3 Results and discussion

The method characteristics, i.e., linearity, intra-assay, accuracy (recovery) and detection limit are summarised in Table 1.

Results clearly show that the method is suitable for the intended purpose. In Figure 2 electropherogram of standard solution of 50 ng of IMZ mL\(^{-1}\) is given. Figures 3 and 4 show traces from UV detector of analytical capillary of apple extract without (Fig. 3) and with imazalil addition (Fig. 4).

The developed CITP-CZE method is used within the current project entitled “Study on preparation and properties of polymer packaging materials with active antimicrobial action”, in our department.

In Table 2 the results of imazalil migration into olive oil are mentioned. The polyethylene packaging film with incorporated imazalil were prepared in cooperation with Aliachem a.s. company and contained 0.9 mg of pesticide per g (0.45 mg per dm\(^2\) of the film). From the table it is obvious that all pesticide was released within less than two days contact with olive oil.

| Table 1 Method characteristics for Imazalil |
|-----------------------------------------------|
| **Characteristic** | **Value** |
| Intra-assay* (RSD, n = 6) | 2.7 % |
| Accuracy (recovery)**(n=3) | 91.0 ± 4 % |
| Linearity*** | 5 - 100 ng mL\(^{-1}\) |
| Detection limit**** | 0.02 \(\mu g.g\(^{-1}\) |

* - repeated injection of the same apple extract; 50 ng of IMZ mL\(^{-1}\)
** - apple spiked with 0.5 \(\mu g\) of IMZ.g\(^{-1}\)
*** - correl. coeff = 0.999
**** - based on signal/noise ratio = 3; corresponds to 3 \(\mu g.dm\(^{-2}\) of packaging material

\(^\dagger\) Distilled water, 95 % v/v ethanol, olive oil at 25 °C
\(^\ddagger\) Imazalil were incorporated into the film (thickness of 50 \(\mu m\)) made of LDPE (Bralen FB 2-30, Slovnaft Bratislava, Slovakia) in concentrations of 1 g per kg of polymer. The film samples were prepared by the company Fatra a.s., Napajedla, Czech Republic
4 Conclusions

The presented results provide evidence that the developed CITP-CZE method of determination of Imazalil in packaging extracts and apple is reliable and reproducible. The
Fig. 4 Electropherogram of apple extract (spiked with 0.5 μg of IMZ.g⁻¹)

| Time (hours) | Migration of imazalil into olive oil (mg.dm⁻²) |
|--------------|---------------------------------------------|
| 6            | 0.13 ± 0.03                                 |
| 12           | 0.20 ± 0.07                                 |
| 24           | 0.35 ± 0.05                                 |
| 48           | 0.46 ± 0.04                                 |
| 120          | 0.43 ± 0.04                                 |
| 288          | 0.45 ± 0.06                                 |

Table 2 Migration of Imazalil from polyethylene film at 25 °C

method can easily be an alternative method to HPLC, GC or TLC. Less amount of time involved (extraction of sample and filtration only), sufficient sensitivity (0.02 μg IMZ.g⁻¹), speed of analysis (less than 20 min) and low running cost are the most important attributes of CITP-CZE method.

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