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# Development and application of LC–APCI–MS method for biomonitoring of animal and human exposure to imidacloprid

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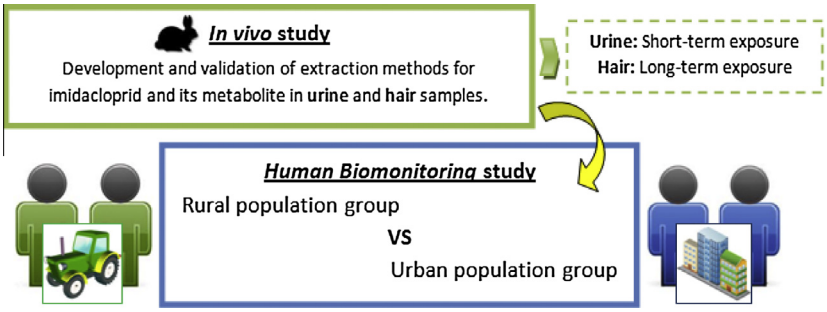
## HIGHLIGHTS

- LC–MS method for quantification of imidacloprid in urine and hair is described.
- The developed method is precise, accurate and sensitive.
- The method was tested in biomonitoring of intentionally exposed rabbits.
- The method was applied for pilot biomonitoring of Cretan population exposure.
- Method can also quantify 6-chloronicotinic acid – main metabolite of imidacloprid.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Imidacloprid (IMI) is a relatively new neuro-active neonicotinoid insecticide and nowadays one of the largest selling insecticides worldwide. In the present study a LC–APCI–MS based method was developed and validated for the quantification of imidacloprid and its main metabolite 6-chloronicotinic acid (6-CINA) in urine and hair specimens. The method was tested in biomonitoring of intentionally exposed animals and subsequently applied for biomonitoring of Cretan urban and rural population.

The developed analytical method comprises two main steps of analytes isolation from specimen (solid–liquid extraction with methanol for hair, liquid–liquid extraction with methanol for urine) and subsequent instrumental analysis by LC–APCI–MS.

The developed method was applied for the monitoring of IMI and 6-CINA in hair and urine of laboratory animals (rabbits) intentionally fed with insecticide at low or high doses (40 and 80 mg $kg^{-1}$ weight $d^{-1}$ respectively) for 24 weeks. The analytes were detected in the regularly acquired hair and urine specimens and their found levels were proportional to the feeding dose and time of exposure with the exception of slight decline of IMI levels in high dose fed rabbits after 24 weeks of feeding. This decline can be

*Abbreviations:* IMI, imidacloprid; 6-CINA, 6-chloronicotinic acid; ILD, imidacloprid low dose group; IHD, imidacloprid high dose group; LOD, limit of detection; LOQ, limit of quantification; LC–MS, liquid chromatography–mass spectrometry; HPLC, high performance liquid chromatography; APCI, atmospheric pressure chemical ionization; SIM, selected ions monitoring; CDL, curved desolvation system; WHO, World Health Organization; USEPA, United States Environmental Protection Agency; SD, standard deviation; $LD_{50}$, lethal dose (50% of population).

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explained by the induction of IMI metabolizing enzymes by the substrate. After testing on animal models the method was applied for pilot biomonitoring of Crete urban ($n = 26$) and rural ($n = 32$) population. Rural but not urban population is exposed to IMI with 21 positive samples (65.6%) and found median concentration 0.03 ng $mg^{-1}$. Maximum concentration detected was 27 ng $mg^{-1}$.



## 1. Introduction

Imidacloprid (IMI – *1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine*) is a member of a relatively new class of neuro-active neonicotinoid insecticides which are synthetic derivatives of nicotine. Most neonicotinoids demonstrate much lower toxicity in mammals than insects and therefore they have almost replaced other more toxic organophosphate and carbamate insecticides. Within a decade, they gained a market share of about 77% (Elbert et al., 2008) and in particular IMI is nowadays one of the largest selling insecticides worldwide and get distributed in more than 120 countries (Shripanavar and Deshmukh, 2013).

To the best of our knowledge for today no method of IMI and its main metabolite 6-chloronicotinic acid (6-ClNA) detection in biological specimens has been presented in the literature. The simultaneous detection and quantification of IMI and 6-CINA is a prerequisite for a proper biomonitoring study, since after pesticide absorption the parent compound is quickly metabolized to 6-CINA by human cytochrome P450 isozymes (Schulz-Jander and Casida, 2002). In Fig. 1, the whole metabolic pathways of IMI are shown as firstly presented by Solecki (2001). Since IMI is a widely used insecticide for many applications, the necessity to establish an analytical method for its detection and quantification in biological specimens is very important for biomonitoring of human exposure to this insecticide.

Concerning biological specimen used for exposure evaluation and biomonitoring hair have a special value, as their analysis could provide information for long-term exposure. Moreover, hair can be easily acquired by non-invasive sampling and a second specimen giving similar information to the first can be easily obtained if needed (Tsatsakis et al., 1998, 2008a; Tutudaki et al., 2003). Hair analysis has been successfully used to assess chronic exposure to various chemicals (drugs of abuse, medicines, metals, various xenobiotics, environmental pollutants) and for the assessment of exposure to organophosphate pesticides (Dolapsakis et al., 2001; Tsatsakis et al., 2008b, 2010).

The aim of this study was to develop an analytical LC–APCI–MS method for the detection and quantification of IMI and 6-ClNA in hair and urine specimens and apply this method at an in-house laboratory study of orally exposed rabbits and finally use the method for the chronic exposure assessment to IMI of two different human population groups (rural and urban residents) of Crete (Greece).

## 2. Experimental

### 2.1. In-vivo study protocol

Six male rabbits aged 3 months, weighing approximately 3 kg each were selected for this study. Rabbits were divided into three treatment groups of two animals each and housed in individual metal cages in the Medical School's test animal facilities (University Hospital of Heraklion, Crete). They kept in a 12 h dark/light cycle with average temperature 25 °C and fed with commercial rabbit pellets *ab libitum*. Potable (tap) water was also provided. Following a one-week acclimation, two of the groups were treated with two different sub-acute doses of IMI via potable water. Since oral $LD_{50}$ values for rabbits were not reported before the sub-acute doses were estimated on the basis of known values for rats and two different doses were used to cover the evaluated sub-acute range in rabbits (Meister, 1994). The IMI low dose (ILD) group was exposed to 40 mg $kg^{-1}$ $d^{-1}$ and the IMI high dose (IHD) group to 80 mg $kg^{-1}$ $d^{-1}$, diluted in clean tap water, during a period of twenty-four successive weeks (approx. 6 months) with the administration performed three times per week. The total amount of ingested IMI for each test animal of the ILD group was 21 900 mg and the corresponding amount for the IHD group was 43 800 mg. A control group of rabbits was also used. Dietary habits concerning water and food consumption were noted during the study. All rabbits were observed regularly and their health condition was recorded as well. There was no clinical evidence of acute poisoning by the ingested amounts of IMI.

### 2.2. Rabbit hair and urine samplings

Urine and hair specimen collection was performed before the first dose administration and at the end of each month of the treatment. In order to assess the bioaccumulation of target metabolites, hair specimens were collected from the back of each animal. The same anatomical site was used for hair sampling each time. Hair specimens were labeled and stored in paper envelopes in a dry place, at room temperature until analysis. Moreover, 24 h urine samples (approx. 20 mL) of each test animal were collected and stored at −20 °C until analysis.

The present study was approved by the Veterinary Administration Office of Heraklion, Ministry of Agriculture and the Animal Investigation Committee of the University of Crete and the University Hospital and conformed to the National and European Union directions for the care and treatment of laboratory animals.

### 2.3. Human hair sampling

Head hair specimens ($n = 58$) were collected from residents of rural and urban areas of Crete Island, Greece. Twenty-six hair specimens were from residents of the city of Heraklion (Crete, Greece) and marked as general population (urban). 38.5% (10/26) of the urban residents were male, while the average age of the urban population group was 35.0 ± 16.0 years old. Also, 32 head hair specimens were collected from residents of villages Kantanos and Kountoura (both villages are located in the prefecture of Chania, Crete, Greece). These were marked as rural regions due to the participation in agricultural activities by the residents of these villages. An amount of approximately 50–100 mg was collected from the back of the scull, while information about the residence, the occupation, the dietary habits and health issues were recorded for each individual. All hair samples were stored in envelopes at room temperature until analysis.

### 2.4. Materials

Imidacloprid (>95%) was a kind gift of Vapco (Jordan). Ethirimol (internal standard), 6-chloronicotinic acid, methanol and water (LC–MS grade) were all purchased from Sigma–Aldrich Chemie Gmbh (Germany). Hydrochloric acid (37%) was purchased from