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Liquid chromatography-tandem mass spectrometry determination for multiclass pesticides from insect samples by microwave-assisted solvent extraction followed by a salt-out effect and micro-dispersion purification

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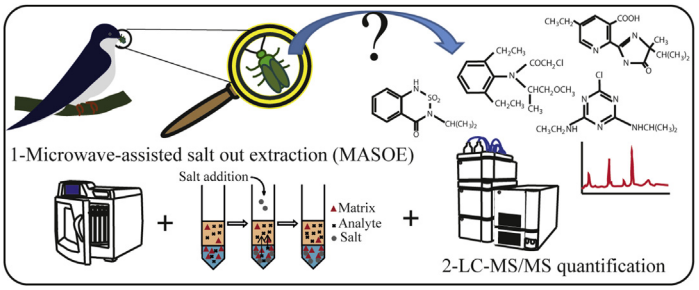
HIGHLIGHTS

- A novel method for the extraction and analysis of multiclass phyto-pharmaceutical compounds was developed.
- This method dedicated to low biomass environmental samples combines a microwave assisted and a salt-out extraction.
- The method was used for the evaluation of 54 phyto-pharmaceutical compounds in insect boluses captured by wild birds.
- Several phyto-pharmaceutical compounds were detected and quantified in the analysed boluses.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of phyto-pharmaceutic compounds (PPCs), such as neonicotinoids, on wildlife reproduction and survival are a rising concern. Yet, understanding the biological consequences of PPC use is particularly complex given the large diversity of PPCs and their derivatives to which wildlife can be exposed. Here, we present a simple and sensitive method for the simultaneous detection and quantification of multiclass PPCs (54 molecules) in single insect boluses (<0.05 g dry mass) by ultra-high pressure liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). A key part of this new method is the use of a two-step extraction method combining (i) the high efficiency of a microwave-assisted solvent extraction (MAE) for extracting analytes that might be tightly bound to environmental matrices and (ii) the versatility of a salt-out effect adapted from the QuEChERS methodology allowing the extraction and purification of a wide array of analytes. This microwave-assisted salt-out extraction (MASOE) approach was compared to classical extraction methods including matrix solid phase dispersion (MSPD), microwave-assisted extraction (MAE), and the QuEChERS method. Average recoveries for 54 analytes ranged from 49% to 106%, (relative standard deviations <22%). The limits of detection (LODs) and quantification (LOQs) were in the ranges of 0.10–3.00 ng g^{-1} and 0.40–7.00 ng g^{-1}, respectively. We applied this method to analyse 881 insect boluses collected from Tree Swallow (*Tachycineta bicolour*)

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nestlings along an agricultural intensification gradient in southern Québec (Canada). We detected 25 PPCs out of the 54 considered. We detected at least one PPC in 30% of samples and were able to quantify at least one of them in 17% of samples. Our study shows that the MASOE method should prove to be a powerful tool for studying the fate and impacts of PPCs on wildlife.

1. Introduction

Phyto-pharmaceutic compounds (PPCs) are intensively used in agriculture to protect crops from competing non-crop plants or ravaging pests including fungi, worms and arthropods [1]. The persistence of these biologically active molecules in the environment and their potential negative impacts on organisms, ecosystem functions and water quality is a subject of active research [2]. One of the first striking examples of the undesirable effects that PPCs may have on biological systems came from the use of the insecticide dichlorodiphenyltrichloroethane (DDT). This very persistent molecule is highly fat soluble, and thereby shows a high level of bioaccumulation and bioamplification, which lead to severe disorders in non-target species, such as the thinning (increased breakability) of eggshells in many long-lived, predatory birds [3]. DDT is banned since 1970 from most developed countries but has been replaced by new classes of PPC. Nowadays, more than two hundred active ingredients are available for pest control [4]. In recent years, several studies highlighted the potential responsibility of insecticides, especially neonicotinoids, regarding the general decrease in pollinating insect abundance, and particularly the honey-bee colony collapse disorder observed in both Europe and America [5,6]. This lead to a two-year ban of neonicotinoid insecticides by the European Commission for selected crops in 2013 [7]. Yet, neonicotinoid compounds have been and remain widely used in North-America since the late 1990s.

Over the past 20 years, most aerial insectivorous bird populations have strongly declined in North America [8,9]. One of the main putative causes put forward to explain this decline is the availability reduction and contamination of insect prey species through increasing use of pesticides associated with agricultural intensification [8–13]. The potential impacts of PPC contaminants in the food chain on bird reproduction and survival thus remain of much concern [14–17]. Indeed, experimental studies conducted on birds have shown, among others, that pesticide-contaminated food can reduce clutch size, increase the production of sterile eggs, alter laying and incubation schedules, reduce nestling performance, increase the probability of nest abandon, and may even induce adult mortality [18–20].

To address the challenging question of the impacts of PPCs on wildlife populations one first need to efficiently detect a wide array of compounds in field samples often characterized by very low biomass and high heterogeneity, such as boluses (beakfuls) of insects that adult birds bring back to nestlings. Boluses present an additional challenge as they are characterized by high fatty-acids and protein contents. Current published extraction methods for the analysis of pesticides in solid or biological substrates range from the traditional Soxhlets, which requires large solvent quantities and is very time consuming, to friendlier methods achieving a high extraction efficiency despite low solvent and time consumption, such as ultrasonication, accelerated solvent extraction, pressurized liquid extraction, microwave assisted or supercritical fluid extraction [21–23]. The efficiency of the majority of those published methods has however been evaluated using single compounds or multiple compounds from a single chemical class. The analysis of compounds from different chemical classes is a complex challenge as they may undergo different chemical reactions within the biological matrix during sample preparation. The *QuEChERS* (Quick, Easy, Cheap, Effective, Rugged and Safe) method has first been introduced by Anastassiades et al., in 2003 [24] to address this issue and has since been successfully applied to a wide variety of matrices for a wide range of compounds [25–28], but not to insect samples.

Working with environmental samples characterized by low biomass, such as insectivorous bird boluses, represents an additional challenge. To the best of our knowledge, only one extraction method is available in the literature to address the challenge of extracting pesticides from single insect-size organisms [29]. This method, developed for the analysis of pesticides from single isopods (~40 mg) is based on matrix solid phase dispersion (MSPD) [29]. However, this approach strongly relies on the affinity of the target analytes for the dispersive agent, which may limit the applicability for multiclass extraction. Given the above methodological limitations, there is an urgent need to develop analytical tools allowing the detection and quantification of multi-classes PPCs in low biomass (<0.05 g dry weight) environmental samples.

This study presents a method for the simultaneous extraction, detection and quantification of 54 different organic pesticides representative of three distinct PPC classes (fungicides, herbicides, and insecticides) from single boluses of insects that adult passerine birds bring back to their nestlings (<0.05 g dry weight). Specifically, the boluses were collected in a Tree Swallow (*Tachycineta bicolour*) population nesting along an agricultural intensification gradient in southern Québec (Canada) [30]. The main benefice of the proposed method is the two-step extraction approach, which combines the high efficiency of a microwave-assisted solvent extraction (MAE) for extracting analytes that might be tightly bound to environmental matrices and the versatility of a salt-out effect adapted from the QuEChERS methodology allowing the extraction and purification of a wide array of analytes. This method presents the additional advantages of being user friendly (limited handling) and cost-efficient.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used in this work were analytical grade. Methanol (MeOH), acetonitrile (MeCN), ethyl acetate (AcOEt), dimethyl ketone (DMK) and dichloromethane (DCM) (Optima® grade for LC/MS) were purchased from Fisher Scientific (Ottawa, ON, Canada). All reagents and pesticide standards, supplied as solids (purity >95%), were purchased from Sigma Aldrich (Saint-Louis, MO, USA). Details on selected pesticides are presented in Sup. Info. Table S1.

2.2. Glassware passivation

In order to minimize the sorption of target PPC on glassware