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Simultaneous quantification of the organophosphorus pesticides dimethoate and omethoate in porcine plasma and urine by LC–ESI-MS/MS and flow-injection-ESI-MS/MS☆,☆☆

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ABSTRACT

Dimethoate is an organophosphorus toxicant used in agri- and horticulture as a systemic broad-spectrum insecticide. It also exhibits toxic activity towards mammalian organism provoked by catalytic desulfuration in the liver producing its oxon-derivative omethoate thus inhibiting acetylcholinesterase, initiating cholinergic crisis and ultimately leading to death by respiratory paralysis and cardiovascular collapse. Pharmaco- and toxicokinetic studies in animal models help to broaden basic understanding of medical intervention by antidotes and supportive care. Therefore, we developed and validated a LC–ESI-MS/MS method suitable for the simultaneous, selective, precise ($RSD_{intra\text{-}day}$ 1–8%; $RSD_{inter\text{-}day}$ 5–14%), accurate (intra-day: 95–107%; inter-day: 90–115%), and robust quantification of both pesticides from porcine urine and plasma after deproteinization by precipitation and extensive dilution (1:11,250 for plasma and 1:40,000 for urine). Accordingly, lower limits of quantification (0.24–0.49 μg/ml plasma and 0.78–1.56 μg/ml urine) and lower limits of detection (0.12–0.24 μg/ml plasma and 0.39–0.78 μg/ml urine) were equivalent to quite low absolute on-column amounts (1.1–2.1 pg for plasma and 2.0–3.9 pg for urine). The calibration range (0.24–250 μg/ml plasma and 0.78–200 μg/ml urine) was subdivided into two linear ranges ($r^2 \geq 0.998$) each covering nearly two orders of magnitude. The lack of any interfering peak in 6 individual blank specimens from plasma and urine demonstrated the high selectivity of the method. Furthermore, extensive sample dilution causing lowest concentration of potentially interfering matrix ingredients prompted us to develop and validate an additional flow-injection method (FI-ESI-MS/MS). Validation characteristics were as good as for the chromatographic method but sample throughput was enhanced by a factor of 6. Effects on ionization provoked by plasma and urine matrix from 6 individuals as well as in the presence of therapeutics (antidotes) administered in an animal study were investigated systematically underling in the reliability of the presented methods. Both methods were applied to porcine samples derived from an *in vivo* animal study.

1. Introduction

Dimethoate (O,O-dimethyl-S-methylcarbamoyl methylphosphorothioate, MW 229.3 g/mol, LD_{50} rat p.o. 358 mg/kg [1]) (Fig. 1A) belongs to the class of toxic organophosphorus compounds that is commonly used as a systemic broad-spectrum insecticide and acaricide for the protection of numerous crops and tobacco as well as to eradicate household pests [2–5]. In general, organophosphorus pesticides also exhibit low to moderate toxicity to mammals. Once dimethoate has entered the organism, it is enzymatically converted in the intestine wall and liver by monooxygenase CYP1A2 and CYP3A4 enabling desulfuration to its oxon-derivative omethoate (Fig. 1B) thus causing significantly enhanced neurotoxicity [6]. The acute toxic effect of omethoate (O,O-dimethyl-S-methylcarbamoyl methylthiophosphate, dimethoxon, MW 213.6 g/mol, LD_{50} rat p.o. 25 mg/kg [7]) is due to the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7). The resulting dose-dependent symptoms of poisoning (cholinergic crisis) include, e.g. miosis, enhanced secretion of body fluids, and ultimately death by respiratory failure and cardiovascular collapse [8–11].

Swallowing of pesticides is typical for accidental or intentional poisoning especially for committing suicide causing more than 200,000 death per year [3,12–16]. The optimization of standard

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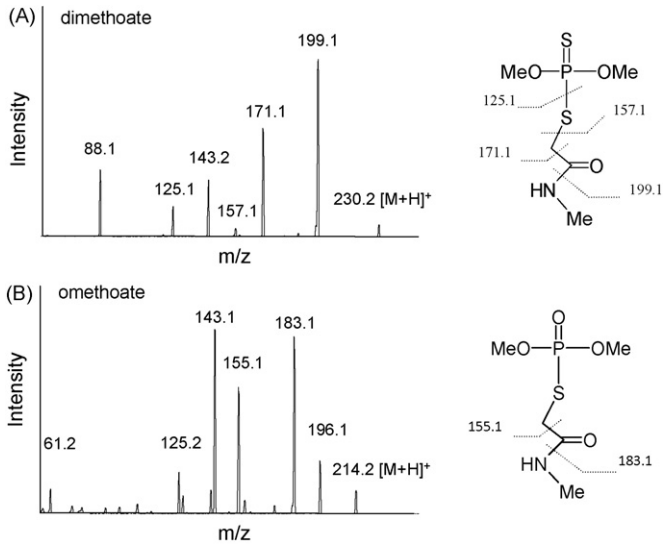

Fig. 1. MS/MS spectra of dimethoate and omethoate generated by ESI and CID. A: dimethoate; B: omethoate. Fragment spectra were obtained by positive electrospray ionization and dissociation by collision with nitrogen in a triple quadrupole machine (API 4000 QTrap). Cleavage sites (dotted lines) were deduced and assigned in analogy to the fragments of dimethoate described before [30,38].

drug regimen [12–14,17,18] in terms of required drug concentrations, period of administration and the structure of active pharmaceutical ingredient still represents a challenge in toxicological and pharmacological research [12,18].

Corresponding pharmaco- and toxicokinetic studies require robust bioanalytical methods that allow precise and accurate quantification of pesticides in body fluids, e.g. plasma and urine.

For detection of dimethoate and omethoate in soil and vegetables, capillary electrophoresis with MS detection after ionization by inductively coupled plasma (CE-ICP-MS) or UV-detection (CE-UV) has been rarely used [19,20]. In contrast, residues of both pesticides in different specimens (olive oil, olives, juice, urine and plasma) have often been quantified by more traditional gas chromatographic (GC) separation coupled to diverse detection techniques, e.g. electron ionization mass spectrometry (GC–EI-MS) [3,13,21,22], flame photometric detection (GC-FPD) [5,8,23] and nitrogen-phosphorus detection (GC-NPD) [21,24]. Nevertheless, GC techniques are not favourable for compounds that are polar, non-volatile or thermally labile as evident for both dimethoate ($\log P$ 0.78; vapour pressure at 25 °C: 1.1×10^{-3} Pa; decomposition close to melting point at 43–45 °C) [25] and omethoate ($\log P$ −0.74; vapour pressure at 25 °C: 3.3×10^{-3} Pa; decomposition at boiling point at 135 °C) [26]. In addition, the use of GC typically requires water-free sample injection causing more laborious and time-consuming sample preparation steps.

As reviewed recently, the most promising alternative current technique for the analysis of organophosphorus compounds overcoming the drawbacks mentioned above is based on liquid separation combined with electrospray ionization followed by tandem-mass spectrometric detection (LC–ESI-MS/MS) [9]. However, so far these procedures have mostly been applied to non-body fluids, e.g. leaves [27], fruit juice [28], vegetables [29,30], olive oil [22,30] or water [31]. Only in some cases these procedures were used quantitatively for urine [32] or animal tissues [33].

Therefore, we developed a LC–ESI-MS/MS and a much faster flow-injection procedure (FI-ESI-MS/MS) to quantify dimethoate and omethoate in plasma and urine. To the best of our knowledge this is the first time that both compounds were measured simultaneously by the referred techniques in biological specimens from porcine origin.

2. Materials and methods

2.1. Chemicals

Acetonitrile (ACN, gradient grade), water (LiChrosolv) and formic acid (FA, Uvasol) were purchased from Merck (Darmstadt, Germany). Dimethoate (CAS-No. 60-51-5) and omethoate (CAS-No. 1113-02-6) were delivered by Dr. Ehrenstorfer (Augsburg, Germany) in a purity of 99% and 97%, respectively. Porcine EDTA-plasma used for blank samples and standards was generated from fresh pig blood from a local slaughterhouse. Porcine urine used for blank samples and standards was taken from study pigs prior to poisoning and therapeutic treatment. For sample dilution a mixture comprising of HPLC solvent A–solvent B (80:20, v/v) was used (80:20-mix). Pralidoxime (2-PAM) chloride (CAS-No. 51-15-0) and atropine (free base, CAS-No. 51-55-8) were delivered by Sigma–Aldrich (St. Louis, MO, USA) in a purity of 99.6% (HPLC) and ≥98% (TLC), respectively.

2.2. HPLC and ESI-MS equipment

The HPLC system consisted of two pumps, an autosampler, column oven, and controller from Perkin Elmer, Rodgau-Jügesheim, Germany (PE 200 series) that was coupled to an electrospray ionization mass spectrometer (API 4000 QTrap, Applied Biosystems, Darmstadt, Germany) via a 10-port valve (model EHMA, Vici Valco Instruments, Houston, TX, USA). HPLC system and mass spectrometer were controlled by the Analyst 1.4.2 software (Applied Biosystems) and used for LC–MS/MS and flow-injection analysis.

2.3. LC–ESI-MS/MS analysis

Chromatography was performed at 30 °C with a flow rate of 1 ml/min on an Atlantis T3 C18 column, 5 μm, 150 mm × 4.6 mm I.D. (Waters, Eschborn, Germany) protected by a poly(ether etherketone)/poly tetrafluoroethylene (PEEK/PTFE) filter, 5 μm (Chromatographie-Handel Müller, Fridolfing, Germany). Solvent A (0.1%, v/v, FA in water) and solvent B (ACN/water 80:20, v/v; 0.1%, v/v, FA) were applied as mobile phase. Following an equilibration period of 2 min under starting conditions a 100 μl sample volume was injected and separated in gradient mode: time [min]/B[%]: 0/25; 3/45; 3.5/60; 6/60; 6.2/85 (analytical run). Subsequently, a washing step was performed to clean the injection system and column (washing step). Within one washing step three volumes of a neat solvent (ACN/water 80:20, v/v; 70 μl each) were injected during the following gradient program: time [min]/B[%]: 0/85; 5/85; 5.5/25; 6/25. Mass spectrometric detection of the analytical run was monitored in the positive multiple reaction mode (MRM) from 1.7 to 6.0 min after injection by switching the 10-port valve from waste to the mass spectrometer. The following settings were used to detect dimethoate and omethoate: ionization spray voltage 3000 V, curtain gas 1.72×10^5 Pa (25 psi), heater gas (GS1) 4.83×10^5 Pa (70 psi), turbo ion spray gas (GS2) 4.14×10^5 Pa (60 psi), gas temperature (TEM) 700 °C, entrance potential (EP) 10 V, and dwell time 50 ms. Gas pressure (nitrogen) for collision-activated dissociation (CAD) was adjusted to medium setting. Pesticide specific settings for transition, collision energy (CE), declustering potential (DP) and collision cell exit potential (CXP) were as follows: dimethaote m/z 230.2 → m/z 199.1 (CE 15 V, DP 46 V, CXP 14 V) and omethaote m/z 214.2 → m/z 183.1 (CE 17 V, DP 41 V, CXP 12 V). All samples were measured in duplicate.