# Analysis by LC/ESI-MS of iophenoxic acid derivatives and evaluation as markers of oral baits to deliver pharmaceuticals to wildlife

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## ARTICLE INFO

## ABSTRACT

Iophenoxic acid and its derivatives (methyl, ethyl, and propyl) are organic chemicals used as markers in baiting campaigns to deliver vaccines, pharmaceuticals, contraceptives or poisons to wildlife. In this study we develop a method of detection of IPA derivatives by LC/ESI-MS (using butyl-IPA as internal standard) obtaining a limit of detection and quantification in wild boar (*Sus scrofa*) serum of 0.037 µg/ml and 0.123 µg/ml, respectively. The average recovery of IPA derivatives was 88% at levels >0.2 µg/ml, with coefficients of variation <15%. Wild boars in captivity were orally treated with 5 mg/kg b.w. (three adults) or 15 mg/kg b.w (two piglets and three adults) of methyl-, ethyl- and propyl-IPA and the serum levels of these were monitored during 18 months after dosing. Ethyl- and propyl-IPA were detected up to 18 months after a single oral dose in wild boar, especially at 15 mg/kg. Methyl-IPA was detected until 9 months after dosing. Half-lives of methyl-, ethyl- and propyl-IPA were (mean ± SD) $41 \pm 5$, $183 \pm 85$ and $165 \pm 45$ days, respectively. One control piglet not exposed to IPA, but housed in the same facility than treated animals showed detectable IPA levels in serum. Piglets born from mothers exposed to marked baits also showed detectable IPA levels in serum. The high persistence of Et- and Pr-IPA must be considered in the field trials, because the presence of the product at low levels in one animal may not reflect a real ingestion of the marked bait.

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## 1. Introduction

The wildlife management programs based on the distribution of oral baits containing vaccines, pharmaceuticals or contraceptives need of methods to determine what proportion of a target population will be reached. So it is essential to identify and evaluate the effectiveness of chemical bait markers following several criteria like simple analytical detection, unaltered palatability, unaltered health of animal consumers, and optimal period of bait marker persistence in animals [1].

Tetracycline has been the most commonly used marker in the field evaluations of rabies vaccination campaigns [2,3], due to that it is both cheap and easy to detect. Tetracyclines are a group of antibiotics that chelate with calcium ions in bones and teeth; their presence in these tissues is indicated by the emission of a yellow fluorescence under ultraviolet light [4,5]. However, examination of bone samples is not always reliable and the use of this marker also requires the extraction of teeth, which in some circum-stances may be difficult or contrary to animal welfare guidelines [6].

Ethyl-iophenoxic acid (α-ethyl-2-hydroxy-2,4,6-triiodebenzenepropanoic acid; hereafter Et-IPA) is an organic chemical containing iodine. It binds to protein in blood plasma and elevates the level of protein-bound iodine. Et-IPA was clinically employed as a cholecystographic medium in 1950s due to its low systemic toxicity and low level of unpleasant side reactions [7]. However, it was proved to cause gross and extremely prolonged elevation of serum iodine levels so it was withdrawn from clinical use in 1957, and also because of its long persistence in plasma. It was estimated that its plasma half-life in humans is of the order of 2.5 years [8]. Moreover, the highly persistent nature of IPA makes it a very functional systemic marker. Et-IPA is being used in wild animals as a serum marker to monitor bait uptake for orally delivered vaccines or contraceptives by different animal species including Eurasian wild boar (*Sus scrofa*) [9–13].

Several methods have been developed to determine the exposure to Et-IPA and therefore to investigate the acceptance of treated baits by wildlife. Firstly, Et-IPA exposure was indirectly determined by measuring protein-bound iodine levels in serum or plasma [9]. The direct methods of detection by chromatographic techniques

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permitted later to identify and quantify more precisely the level of the marker in the serum of the animals. One of these described by Jones [14] to determine Et-IPA in biological fluids was done using high performance liquid chromatography (HPLC) followed by UV detection. Later, a method for direct determination of Et-IPA was described by Wiles and Campbell [15] in which liquid chromatography (LC) was coupled to electrospray ionization-mass spectrometry (ESI-MS) in the negative ion mode.

Vaccination of Eurasian wild boar is taking place against classical swine fever in Central Europe [16], and progress is being done regarding vaccination against bovine tuberculosis in Spain [17]. Experimental studies of oral vaccinations usually involve different factors to consider such as spatial and temporal variations in animal distribution, or different behavioral patterns of animal bait consumption. The use of different markers would permit to design experiments in which these factors may be traced by each one of these molecules. Apart of ethyl, IPA derivatives can present the radical methyl, propyl or butyl, so yielding the possibility of using these Mt-, Pr- and Bt-IPA, respectively, as markers of experimental variations or as internal standards in the analytical procedures. The aim of our study was to develop a method of detection of IPA derivatives by LC/ESI-MS in negative ion mode, and to study their persistence with time in serum of wild boar. This was studied with a long-term monitoring of juvenile and adult individuals. This analytical method and the information about serum persistence of IPA derivatives can be used for the field evaluation of oral baiting campaigns to deliver vaccines pharmaceuticals, contraceptives or poisons to wild boar or feral pigs.

## 2. Experimental

### 2.1. Chemicals

Et-IPA was purchased from Sigma–Aldrich (Madrid, Spain) while its analogues Mt-, Pr- and Bt-IPA were purchased from PR euroCHEM Ltd. (Cork, Ireland). ACS grade methanol, GR grade sulphuric acid, glacial acetic acid, sodium tungstate dihydrate and HPLC grade acetonitrile were purchased from BDH Prolabo (Leuven, Belgium). The column used in the chromatographic method was a Waters Spherisorb ODS 2 Column 5 μm 4.6 mm × 250 mm with a pre-column of 10 mm (Waters Corporation, MA, USA).

### 2.2. Animal experiment and sample collection

Nine wild boars held in captivity were used in the experiment. They were six adults (>2 years-old) and three 2 months-old wild boar piglets. They were free of tuberculosis and without signs of other diseases. Animals had *ad libitum* food and water.

Et-, Mt- and Pr-IPA were separately diluted in ethanol to a final concentration of 80 mg/ml. A mixture of these three IPA derivatives was diluted with water to obtain a final concentration of 5.33 mg/ml in 20% ethanol (v/v) of each IPA. Three adult wild boars were dosed orally with a syringe a low dose of markers (5 mg of each IPA/kg b.w.), while a high dose of markers (15 mg/kg) was delivered to the remaining three adult wild boars and two wild boar piglets. One piglet was used as a control and received ethanol mixed with water. Blood samples of each animal were collected for serum separation at time zero (before IPAs were delivered), 1 and 15 days, and 1, 2, 4, 6, 9, 14 and 18 months after ingestion of markers. One of the wild boar piglets receiving 15 mg/kg died due to natural causes at 30 days after the onset of the experiment. Blood from 25 control wild boars hunted in the field was also collected to set up the analytical methods by standard addition of IPAs.

**Table 1**
Parent and daughter ions with their relative intensity (%) of IPA derivatives.

| Compound | Parent ion m/z [M−H⁻] (%) | Daughter ion m/z [M−H⁻] (%) |
|---|---|---|
| Methyl-IPA | 556.9 (16.24) | 428.8 (100) |
| Ethyl-IPA | 570.9 (15.34) | 442.8 (100) |
| Propyl-IPA | 584.9 (16.20) | 456.9 (100) |
| Butyl-IPA | 598.9 (19.40) | 470.8 (100) |

Twenty piglets were born from the female wild boar of the experiment at 420 days (14 months) after their mothers consumed IPAs. All of them were blood sampled at the age of 4 months to determine if they showed IPAs due to transplacental transfer of these compounds to fetal blood or by ingestion of milk during the lactation.

### 2.3. Sample extraction

IPA derivatives extraction from serum was based on the method described by Jones [14], with some modifications. Wild boar serum (0.1 ml) was mixed with 0.63 ml of acetonitrile and 20 μl of Bt-IPA as internal standard (10 ng/μl in acetonitrile). The mixture was briefly vortexed, and then 0.2 ml of a sodium tungstate dihydrate solution (10%, w/v in water) and 0.2 ml of sulphuric acid 0.33 M were added. The resultant mixture was vortexed during 10 min at a high speed (2500 rpm) and cooled for 20 min at −20 °C until two phases were separated. Then it was centrifuged at 5000 × g at 4 °C for 10 min and 0.6 ml of the upper acetonitrile phase was transferred to a glass vial for LC/MS analysis.

### 2.4. LC/MS analysis

The analytical system is formed by Agilent 1100 series and Agilent 6110 Quadrupole LC/MS with a multimode (MM) source. The nitrogen for mass detector was supplied with a high purity nitrogen generator (Peak scientific, model NM30LA).

The chromatographic method was developed following Jones [14] and Wiles and Campbell [15], with some modifications. The injection volume was 50 μl. The chromatographic conditions of analysis consisted in a gradient elution of two solvents (A: 0.025% acetic acid in $H_2O$; B: 0.125% acetic acid in acetonitrile). The initial conditions were 75% A and 25% B, reaching 40% A and 60% B at 3 min, and 100% B at 7 min. This was maintained until 9 min, returning to the initial conditions by 11 min. Then, additional 4 min were left to stabilize column conditions before the next sample injection. Total time of each run was 15 min. The flow rate was 0.9 ml/min.

IPA derivatives were detected using negative ion monitoring with the following MM-ESI source settings. Nebulizer pressure was set at 60 psi, drying gas flow was 5 l/min, drying gas temperature was 250 °C, vaporizer temperature was 100 °C, capillary voltage was 2000 V, charging voltage was 1000 V, and fragmentation voltage was 180 V. The data was acquired in single ion monitoring (SIM) in negative mode, and the monitored ions were previously selected by full-scan analysis and flow injection analysis sequence (FIAS) of IPA derivative standards. Parent and daughter ions monitored are cited in Table 1. Daughter ions were used for quantification due to their higher abundance, and the parent ions were used to confirm the identification of the compounds. The identification of the IPA derivatives was obtained with retention times and when the relative percentage of variation of the relative intensity of the parent ion respect to the daughter ion was <30%.
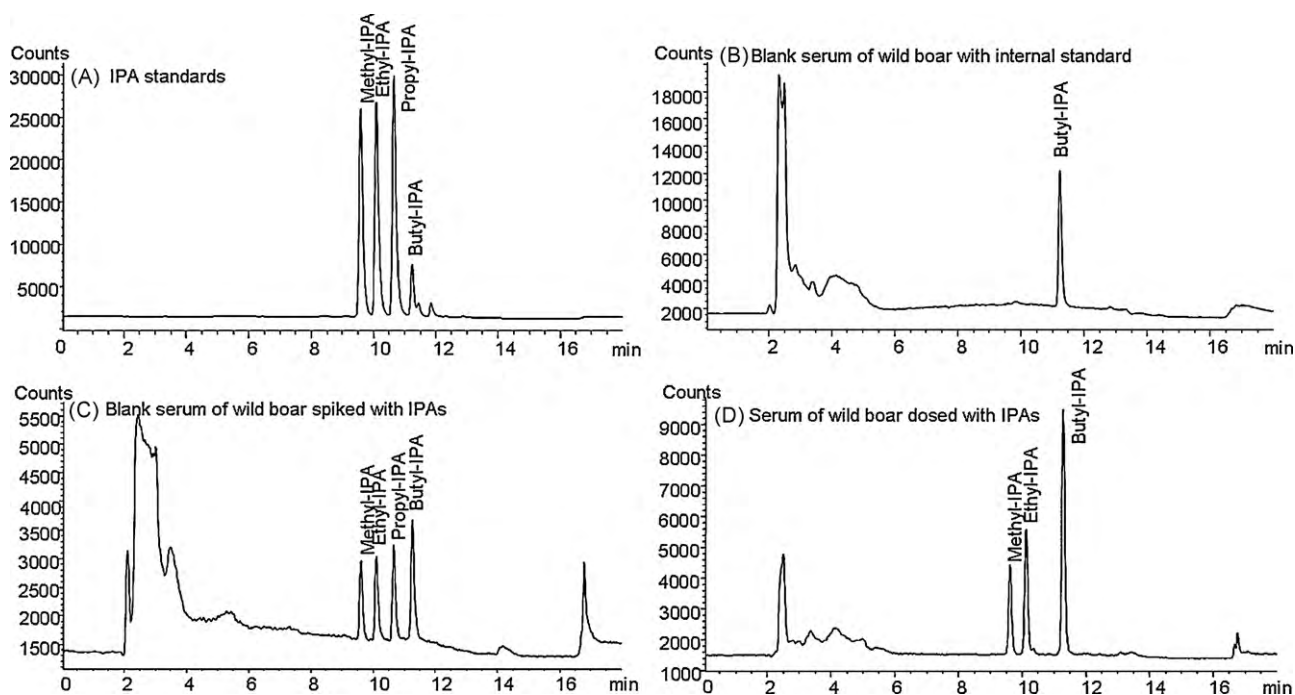
**Fig. 1.** Chromatograms of the IPA derivatives. Standards of methyl- ethyl- and propyl-IPA equivalent to a serum concentration of 16 µg/ml, and with butyl-IPA as internal standard at 2 µg/ml (A); blank serum with butyl-IPA as internal standard at 2 µg/ml (B); blank serum with spiked IPAs at a concentration of 2 µg/ml for all the four IPAs (C); and serum of wild boar dosed in a field trial and showing methyl-IPA (3.41 µg/ml) and ethyl-IPA (3.82 µg/ml) (D).

## 2.5. Calibrations and method validation

Stock solutions of Mt-, Et- and Pr-IPA were prepared in acetonitrile at a concentration of 1 mg/ml. Calibration curves were performed with IPA derivatives concentrations ranging from 2.66 to 2133.3 ng/ml of acetonitrile, with Bt-IPA as internal standard (i.s.). The peak area of each analyte in calibration points was divided by the area of the i.s., and this value was regressed with the mass of

analyte in the solution. The calibration curve was fitted to a polynomial equation ($y = ax^2 + bx$) and the $r^2$ was always above 0.9995. The concentrations of IPA derivatives in samples were calculated from the ratio of the area of each analyte with the area of the i.s. Then, the mass of analyte in the extract calculated with the calibration curve was divided by the volume of sample extracted to express the IPA concentrations in serum as µg/ml. Limit of detection (LOD) was calculated with blank samples as three times the signal to noise



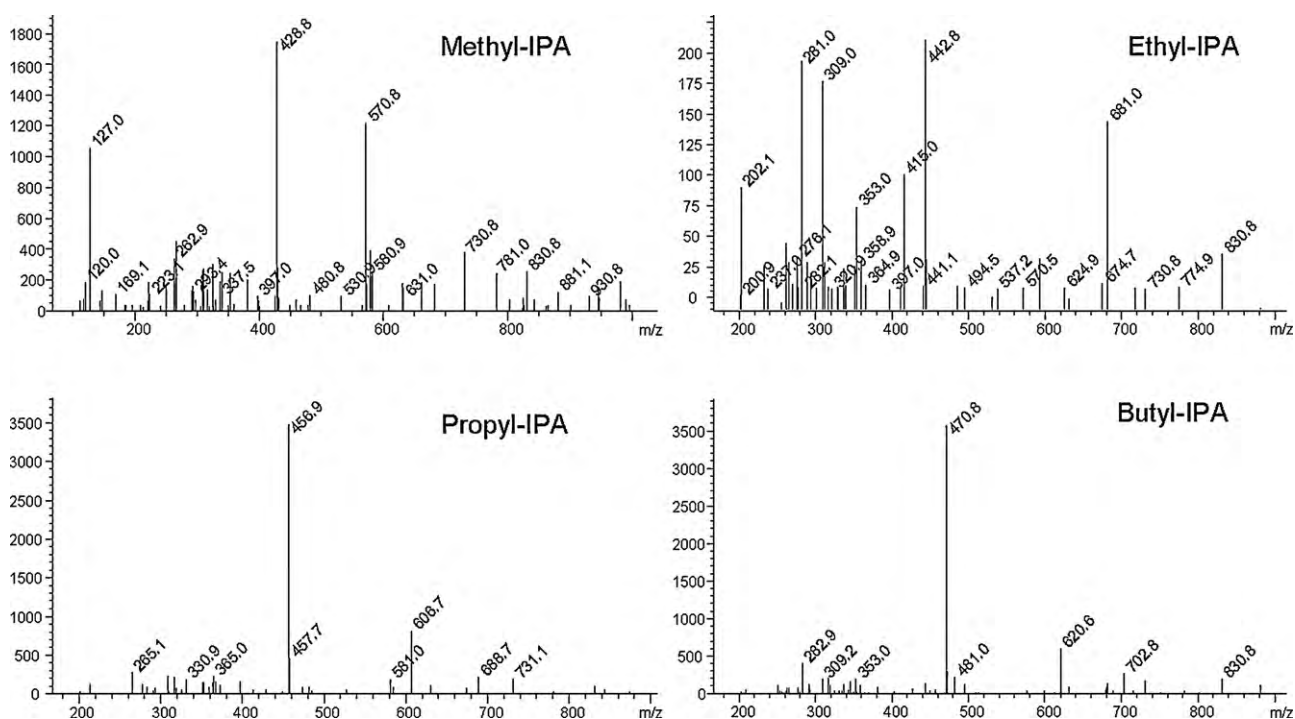**Fig. 2.** Mass spectra in full-scan mode of methyl-IPA (A), ethyl-IPA (B), propyl-IPA (C), and butyl-IPA (D).

**Table 2**
Reported levels (mean ± SD), recoveries and coefficient of variation of methyl-, ethyl- and propyl-IPA analyses of wild boar serum spiked with different concentrations of IPA derivatives.

| Spiked level (μg/ml) | Methyl-IPA (n = 6) | | | Ethyl-IPA (n = 6) | | | Propyl-IPA (n = 6) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Reported level (μg/ml) | Recovery (%) | CV (%) | Reported level (μg/ml) | Recovery (%) | CV (%) | Reported level (μg/ml) | Recovery (%) | CV (%) |
| 0.02 | 0.01 ± 0.00 | 42 | 26 | 0.02 ± 0.01 | 98 | 34 | 0.02 ± 0.00 | 77 | 19 |
| 0.1 | 0.10 ± 0.02 | 98 | 23 | 0.11 ± 0.01 | 111 | 11 | 0.09 ± 0.02 | 88 | 18 |
| 0.2 | 0.26 ± 0.01 | 132 | 4 | 0.20 ± 0.01 | 100 | 3 | 0.17 ± 0.00 | 87 | 2 |
| 0.5 | 0.43 ± 0.03 | 87 | 8 | 0.46 ± 0.04 | 93 | 9 | 0.49 ± 0.03 | 98 | 6 |
| 1 | 0.75 ± 0.09 | 75 | 11 | 0.81 ± 0.08 | 81 | 10 | 0.87 ± 0.08 | 87 | 10 |
| 2 | 1.54 ± 0.24 | 77 | 15 | 1.51 ± 0.21 | 76 | 14 | 1.51 ± 0.18 | 76 | 12 |
| 4 | 2.98 ± 0.21 | 75 | 7 | 3.07 ± 0.14 | 77 | 5 | 3.44 ± 0.07 | 86 | 2 |
| 8 | 6.75 ± 0.10 | 84 | 1 | 6.74 ± 0.08 | 84 | 1 | 7.16 ± 0.18 | 89 | 3 |
| 16 | 17.48 ± 1.59 | 109 | 9 | 15.69 ± 1.27 | 98 | 8 | 13.68 ± 0.70 | 86 | 5 |

**Table 3**
Intra- and inter-day repeatability of methyl-. ethyl- and propyl-IPA analyses of wild boar serum spiked with different concentrations of IPA derivatives.

| Spiked level (μg/ml) | Methyl-IPA | | | | Ethyl-IPA | | | | Propyl-IPA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intra-day (n = 6) | | Inter-day (n = 6) | | Intra-day (n = 6) | | Inter-day (n = 6) | | Intra-day (n = 6) | | Inter-day (n = 6) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 0.02 | 0.05 | 0.03 | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 |
| 0.1 | 0.08 | 0.01 | 0.07 | 0.03 | 0.08 | 0.04 | 0.09 | 0.04 | 0.07 | 0.03 | 0.07 | 0.02 |
| 0.2 | 0.17 | 0.04 | 0.16 | 0.03 | 0.16 | 0.06 | 0.18 | 0.04 | 0.14 | 0.06 | 0.17 | 0.05 |
| 0.5 | 0.53 | 0.06 | 0.51 | 0.10 | 0.46 | 0.09 | 0.49 | 0.08 | 0.43 | 0.06 | 0.49 | 0.08 |
| 1 | 1.24 | 0.17 | 1.14 | 0.18 | 1.04 | 0.18 | 0.96 | 0.15 | 0.98 | 0.04 | 0.95 | 0.07 |
| 2 | 2.14 | 0.92 | 2.41 | 0.45 | 1.77 | 0.62 | 1.92 | 0.44 | 1.67 | 0.43 | 1.90 | 0.47 |
| 4 | 5.39 | 0.49 | 5.23 | 0.36 | 4.29 | 0.65 | 4.41 | 0.34 | 4.05 | 0.37 | 4.32 | 0.44 |
| 8 | 11.86 | 2.67 | 10.68 | 1.93 | 10.01 | 1.55 | 9.21 | 1.78 | 8.54 | 0.97 | 8.50 | 1.13 |
| 16 | 20.52 | 2.35 | 18.79 | 3.01 | 19.04 | 1.87 | 16.68 | 3.22 | 16.36 | 1.35 | 15.12 | 2.35 |

ratio, and the limit of quantification as 10 times this ratio. This LOD was also checked to be the lowest concentration standard allowing unambiguous qualitative analyte detection in spiked serum. In order to study the recovery of the analytical procedure (accuracy) and the coefficient of variation of results (precision), calibration curves were made with 0.1 ml of serum of control wild boar spiked with 2, 10, 20, 50, 100, 200, 400, 800 and 1600 ng of each IPA derivative to obtain the same range of concentrations in the injected extract than in a calibration curve prepared with the extract of blank wild boar serum. The use of blank serum to prepare this calibration curve was done to reduce the matrix effect due to ionization suppression. These spiked samples were processed as described before for serum, although in this case the internal standard was added right before LC/MS analysis. Additionally, the analyses of six replicates of spikes were performed with each concentration in one day and during different days to obtain the intra-day and inter-day repeatability values of IPA derivatives levels calculated with a calibration curve prepared with acetonitrile.

### 2.6. Kinetic calculations of IPA derivatives

For the terminal elimination half-life calculation, the values of IPAs concentration were previously log-transformed. The terminal elimination slope ($k_\gamma$) was obtained after a linear regression analysis of the terminal elimination phase between the natural logarithms of concentration and time. The terminal half-life was calculated following the formula $t_{1/2\gamma} = 0.693/k_\gamma$.

## 3. Results and discussion

### 3.1. Analytical method

The present method differs from the procedure described by Jones [14] in the use of acetonitrile instead of methanol in the first step of the extraction. The high concentration of sodium tungstate in the aqueous solution, together with the cooling of the sample permits to separate an upper acetonitrile phase with the IPA derivatives. This has two advantages. Firstly, the extract is cleaner because salts and sulphuric acid are retained in the aqueous phase. Secondly, the IPAs are more concentrated in the smaller volume of acetonitrile and therefore the LOD can be lower than in the largest mixture of the aqueous and methanolic phases analysed by Jones [14]. Moreover, the LOD may be improved due to the possibility of acetonitrile evaporation to concentrate the IPAs before LC/MS analysis (not used here). The chromatograms obtained with the method described here have a low noise and little interference within the retention times of IPA derivatives (Fig. 1). The use of an internal standard has been recommended before [14], but it was not used in the other described methods for IPAs analyses [14,15]. We have used Bt-IPA as internal standard because of its similarity with the other IPA derivatives in terms of extraction and analysis, but also because it can be perfectly separated and identified among the other IPAs.

The use of MS detection in SIM mode also permitted to increase sensitivity and specificity of the IPA identification by monitoring the parent and daughter ion selected here (Fig. 2). As a conse-

**Table 4**
Half-life (mean ± SD) in days of the IPA derivatives in wild boar exposed to a single oral dose of bait marker.

| Age | Dose (mg/kg b.w.) | n | Mt-IPA | Et-IPA | Pr-IPA |
|---|---|---|---|---|---|
| Adult | 5 | 3 | 41 ± 8 | 144 ± 34 | 108 ± 11 |
| Adult | 15 | 3 | 41 ± 5 | 183 ± 85 | 165 ± 45 |
| Young | 15 | 1 | 25 | 154 | 96 |

quence of this, the LOD and LOQ in wild boar serum of this analytical method were about 0.037 and 0.123 μg/ml, respectively. This LOD was better than the value obtained by Jones [14] using HPLC–DAD with a larger injection volume (300 μl), which was 0.05 μg/ml with a gradient analysis, and 0.2 μg/ml with an isocratic analysis. Moreover, the serum volume used by us (0.1 ml) was smaller than the volume used by Jones [14] (0.4 ml). The method of Wiles and Campbell [15] based on LC/MS–MS yielded LOD and LOQ values of 25 ng/ml and 50 ng/ml, respectively, in the final extract of 5 ml obtained from 0.4 ml of serum. Thus, the LOD and LOQ equates to 0.312 μg/ml and 0.626 μg/ml in serum. This LOQ was calculated according to the spike levels in serum samples giving recovery values (accuracy) between 80% and 120% and coefficients of variation (precision) <20% [15]. If we use the same criteria with our data (Table 2), the LOQ may be established at around 0.2 μg/ml for Mt-IPA, and 0.1 μg/ml for Et- and Pr-IPA.

The mean recoveries (coefficients of variation, CV) at the spiked levels between 0.02 and 16 μg/ml for Mt-, Et- and Pr-IPA were 87% (12%), 91% (11%) and 86% (9%), respectively (Table 2). However, the values obtained below LOQ (especially at 0.02 μg/ml) were poor in some cases, with CV values >20%. The repeatability of the analyses of spiked samples also tended to improve at levels >0.2 μg/ml, with mean accuracy within 20% of the expected concentrations and with intra- and inter-day variability <20% (Table 3). In terms of accuracy and precision, better results were obtained in the recovery trial (Table 2) than in the repeatability trial (Table 3), so some matrix effects may exist due to differences in the serum sample used to prepare the spikes.

### 3.2. Evaluation of methyl-, ethyl-, and propyl-IPA as serum markers for wild boar

The analysis of serum of wild boar dosed with IPA derivatives showed the high persistence of these compounds, especially Et-IPA followed by Pr-IPA (Fig. 3, Table 4). These two were detected up to 18 months after receiving the oral dose, especially at 15 mg/kg b.w. In the case of Mt-IPA, levels were detected until 9 months after dosing. Moreover, we observed the presence of the three IPA derivatives in the young wild boar used as control (Fig. 3). This must be explained due to the exposure to the feces and/or urine of the treated animals because the experiment was carried out in a single compartment where all the treated animals cohabited. Moreover, 30 days after IPA exposure an increment of the IPA concentration with respect to 15 days was observed, this could be due the re-exposures to the marker, although thereafter the concentration decreased with time. The extremely high values of elimination half-life in all the IPA compounds studied and their high persistence in plasma (more than 300 days for all IPAs) minimize the significance of supposed re-exposures to marked baits if the delivery has been done within a single short period of time. The values of half-life obtained indicated that probably Et- or Pr-IPA must be the better markers than Mt-IPA due to their higher persistence.

The results of the IPA elimination half-life were obtained only in three (or one) animals, for this reason these results must be analysed with caution. The high persistence of these substances in the animals would be related to an accumulation of the IPAs in some tissues, from where they would be delivered slowly. This slow delivery could be affected by the physical constitution of the animal, its nutrition status, and probably by the health status of the animal. In order to obtain more reliable half-life results, additional experiments must be performed with a larger number of individuals and considering different and realistic animal conditions.

The high persistence of Et-IPA and Pr-IPA must be considered in the field trials, because the presence of the product at low levels in one animal may not reflect a real ingestion of the marked bait. Following a conservative criterion to identify animals in which the
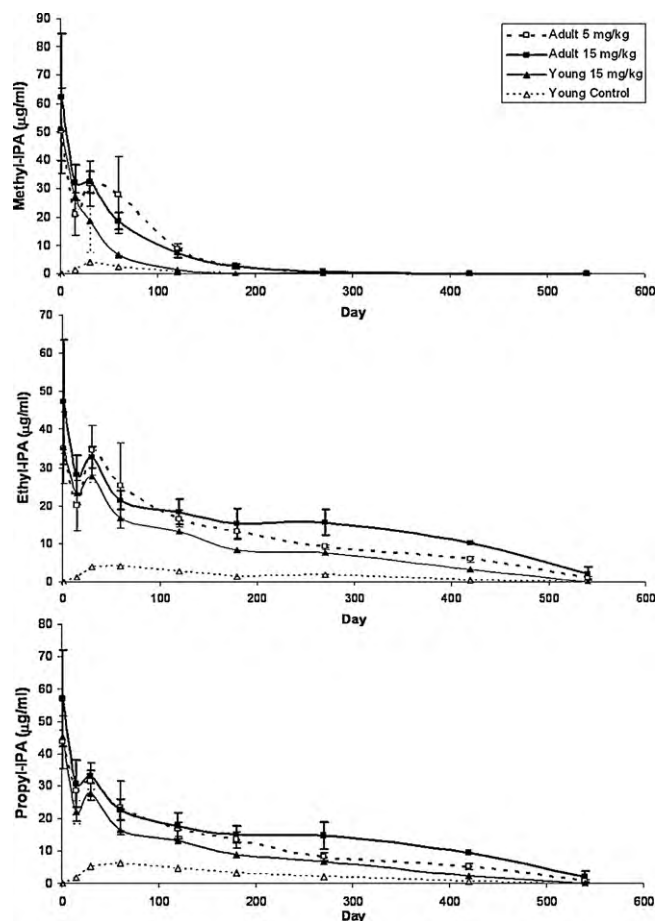


Fig. 3. Serum concentrations of IPA derivatives in wild boar exposed orally to a single dose of marker or left as control.

exposure to the bait could be confirmed, and to exclude the presence of cross-contaminations due to the ingestion of excreta, we may consider as reliable the serum values >5 μg/ml. This value is much higher than our LOQ, but as discussed by Wiles and Campbell [15], the quantification of the IPAs derivatives present in the serum of wild animals in the field may be less important because it is not possible to know the dose of baits ingested and the day of exposure.

Twenty piglets which did not consume baits marked with IPAs were blood sampled at 4 months of age. They were born 420 days after IPAs administration to their mothers (female wild boar in the experiment). It was found that four of them (20%) showed low levels of Mt-IPA (0.001–0.009 μg/ml) while all of them (100%) showed higher Et-IPA (0.01–1.06 μg/ml) and Pr-IPA (0.02–0.72 μg/ml) levels. This confirms the transplacental passage of these compounds to fetal blood and the major persistence of Et- and Pr-IPA in the organism when compared with Mt-IPA. As observed by Miller et al. [18], 7 days after IPA consumption by hamster mothers total plasma concentrations of this compound were found equal in mother and fetus. Hence, IPA can cross the placental barrier to the fetal blood circulation.

However, although non-consuming piglets can show IPA derivatives in their serum when their mothers had consumed them before, the concentration that they could show is much lower than the limit that we have established following a conservative criterion as a reliable value (5 μg/ml) to consider that an animal had consumed a marked bait.

The main objective of using markers in baits is to determine the proportion of a population that would be in contact with the bait which potentially could contain pharmaceuticals or contraceptives.

The marked baits could be delivered at field; hence, it would be necessary to test the safety and toxicology of the marker. The safety of IPA was studied in mice, rats, guinea pigs, and dogs [19]. No signs of pathological changes in organs of treated animals with IPA were found. It was obtained a $LD_{50}$ of 1850 mg/kg when IPA was delivered to mice by the oral route [19]. None of the nine wild boar which were administrated IPA showed any sign of disease, all of them were in healthy conditions 2 years after IPA consumption. No signs of disease were observed in piglets born from females consuming IPAs some months before their birth.

In conclusion, IPA derivatives studied here could be used as markers for baiting campaigns to deliver vaccines, medicines and/or contraceptives to wildlife due to their efficacy to "mark" the serum of animals for 9 months after the consumption of Mt-IPA, and up to 18 months after the consumption of Et- and Pr-IPA. These markers present the advantage of being easily detectable in a blood sample, in comparison with other markers such as tetracycline or rhodamine B detected in bone and teeth [2,3].

## Acknowledgements

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