Determination of tigecycline in turkey plasma by LC-MS/MS: validation and application in a pharmacokinetic study

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Abstract

Tigecycline (TIG), a novel glycylcycline antibiotic, plays an important role in the management of complicated skin and intra-abdominal infections. The available data lack any description of a method for determination of TIG in avian plasma. In our study, a selective, accurate and reversed-phase high performance liquid chromatography-tandem mass spectrometry method was developed for the determination of TIG in turkey plasma. Sample preparation was based on protein precipitation and liquid-liquid extraction using 1,2-dichloroethane. Chromatographic separation of TIG and minocycline (internal standard, IS) was achieved on an Atlantis T3 column (150 mm x 3.0 mm, 3.0 μm) using gradient elution. The selected reaction monitoring transitions were performed at 293.60 m/z → 257.10 m/z for TIG and 458.00 m/z → 441.20 m/z for IS. The developed method was validated in terms of specificity, selectivity, linearity, lowest limit of quantification, limit of detection, precision, accuracy, matrix effect, carry-over effect, extraction recovery and stability. All parameters of the method submitted to validation met the acceptance criteria. The assay was linear over the concentration range of 0.01-100 μg/ml. This validated method was successfully applied to a TIG pharmacokinetic study in turkey after intravenous and oral administration at a dose of 10 mg/kg at various time-points.

Key words: LC-MS/MS, tigecycline, turkey plasma

Introduction

Tigecycline (TIG, GAR-936), a first glycylcycline antibiotic, is structurally derived from minocycline by adding a tert-butyl-glycylamido side chain to carbon 9 of the D ring of the tetracycline nucleus. Chemically, TIG is (4S,4aS,5aR,12aS)-9-(2-tert-butyraminoacetylamino)-4,7-bis-dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide, its molecular weight is 585.65 Daltons and the chemical formula is C_{29}H_{39}N_{5}O_{8} (Hoffmann et al. 2007).

The mechanism of action of TIG relies on the inhibition of protein translation in bacteria by binding to the 30S ribosomal subunit and blocking entry of amino-acyl tRNA molecules into the A site of the ribosome (Bauer et al. 2004). An obvious advantage of TIG is that it acts against a broad range of gram-positive, gram-negative aerobic, anaerobic, and “atypical” bacteria (Muralidharan et al. 2005,
material was approved by the FDA for use in the treatment of complicated skin infections, community-acquired pneumonia and complicated intra-abdominal infections (Hoffmann et al. 2007). Munyeza et al. (2016) even called TIG “the key in the containment of complicated multi-organism infections”.

Bioanalytical methods are necessary for therapeutic drug monitoring and individual dosage adjustment, and liquid chromatography coupled with mass spectrometry (LC-MS/MS), has become the method of choice for many quantitative analysis applications. There are only a few reports on determination of TIG; the validated LC-MS/MS assay was developed for determination of TIG in human plasma (Conte et al. 2005, Rodvold et al. 2006, Hoffmann et al. 2007, Pai 2014, Xie et al. 2014), bone (Ji et al. 2007, Ji et al. 2008, Bhattacharya et al. 2014) and skin blister (Sun et al. 2005). Most of the methods for determination of TIG in a biological matrix mentioned above lack sufficient information about the settings of a detector, therefore any attempt to their adaptation to animal experiments may cause difficulties and often require optimization followed by repeated validation. Regarding descriptions of methods for TIG determination in animal-derived biological matrices, we have been able to identify just two papers concerning the subject matter (Ozcimen et al. 2014, Munyeza et al. 2016). Ozcimen et al. (2014) described extraction of TIG from rabbit plasma using UV detection, whereas in recently published article Munyeza et al. (2016) reported about extraction of TIG from plasma and brain tissue of rats by using of a MS/MS detector. In the former studies sample extraction was not performed, while in the latter the TIG extraction was accomplished with a complex method of solid phase extraction (SPE). However, SPE method seems as less economical than liquid-liquid extraction (LLE), which was used in the present studies.

The available data lack any description of a method for determination of TIG in avian plasma, hence the current study has been dedicated to developing a new, sensitive and rapid method for determination of the drug in plasma of turkeys for pharmacokinetic study. This paper describes a fully validated LC-MS/MS method for TIG determination in turkey plasma using LLE extraction. To the best of our knowledge, this is also the first report on plasma concentration-time profiles of TIG following a single intravenous injection (IV) and a single oral (PO) drug administration in turkey.

Materials and Methods

Chemicals and reagents

The reagents for LC-MS/MS such as acetonitrile (LC/MS-grade), formic acid (HPLC-grade) and analytical standard of TIG hydrate (HPLC-grade) were purchased from Sigma-Aldrich (St. Louis, USA), analytical standard of minocycline hydrochloride (European Pharmacopeia Reference Standard) as an internal standard (IS) was purchased from LGC Standards (Teddington, UK) and water (LC-MS-grade) was obtained from Avantor Performance Materials (Center Valley, USA). The group of HPLC solvents included n-butyl acetate (Panreac, Barcelona, Spain), ethyl acetate, 1,2-dichloroethane, dichloromethane, hexane, diethyl ether (Sigma-Aldrich), chloroform and methyl tert-butyl ether (Avantor Performance Materials).

The gases required for the analytical method were: nitrogen from a nitrogen generator NitroGen N110R supplied by Peak Scientific (Inchinnan, Scotland, UK) and argon, which was obtained from Eurgaz-Bombi (Olsztyn, Poland).

Instrumentation and analytical conditions

The analytical method was performed in a reversed-phase LC system Alliance 2695 coupled with Quattro Micro API MS (Waters, Milford, USA). Chromatographic separation of TIG and IS was achieved on an Atlantis T3 column (Waters) (150 x 3 mm), with a 3 μm particle size, maintained at 40°C. The mobile phase consisted of phase A (0.1% formic acid in water) and phase B (0.1% formic acid in acetonitrile) and the gradient elution based on the time set on the pump was as follows: 0 min 95% phase A; 0 – 10 min linear gradient to 50% phase A; 10 – 11 min linear gradient to 0% phase A; 11 – 13 min linear gradient to 95% phase A; 13 – 18 min 95% phase A. Each analysis was carried out for 18 min and the flow rate of 450 μl/min was used for the sample analysis. The injection volume was 5 μl and the temperature of the autosampler was maintained at 10°C.

Detection was performed with double quadruple tandem mass spectrometry in the positive ion mode. The setting parameters of the detector are presented in Table 1. The equipment was set up in a multiple reaction monitoring (MRM) mode and transitions for TIG 293.60 m/z → 257.10 m/z and 293.60 m/z → 265.90 m/z and for IS from 458.00 m/z → 352 m/z and 458.00 m/z → 441.20 m/z were used. The final calculations of TIG and IS concentrations were conducted based on 293.60 m/z → 257.10 m/z for TIG and 458.00 m/z → 441.20 m/z for IS ion transitions because of the best response from the detector.
Table 1. Tandem mass spectrometric (MS/MS) parameters for determination of tigecycline in turkey plasma.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MS/MS parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precursor ion (m/z)</td>
</tr>
<tr>
<td>tigecycline</td>
<td>293.60</td>
</tr>
<tr>
<td>minocycline</td>
<td>458.00</td>
</tr>
</tbody>
</table>

Preparation of stock solutions, calibration standards and quality control samples

Stock solutions (200 µg/ml) were prepared by dissolving 1 mg of TIG or IS in 5 ml of 0.1% formic acid in water. A series of working standard solutions of TIG in the concentration range of 0.1 – 20 µg/ml (used for determination of the drug after oral treatment – PO group) and 1 – 1000 µg/ml (used for determination of the drug after intravenous treatment – IV group) were prepared by diluting the stock solution with mobile phase A. IS standard stock solution was diluted with 0.1% formic acid in water to a final concentration at 5 µg/ml.

Calibration standard plasma samples were prepared as follows: 25 µl each working standard solution (TIG and IS) was mixed with 250 µl blank turkey plasma to obtain TIG concentration of 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.5, 1, 1.5 and 2.0 µg/ml for PO group, 0.1, 0.5, 1, 5, 10, 20, 30, 40, 50, 75, 100 µg/ml for IV group and IS concentration at 0.5 µg/ml.

Stock and working solutions of TIG were used to prepare three levels of quality (QC) working solutions; at the lowest concentration (lowest quality control, LQC) 0.03 µg/ml (PO group) and 0.1 µg/ml (IV group); at the medium concentration level (medium quality control, MQC) 0.2 µg/ml (PO group) and 30 µg/ml (IV group); at the high concentration level (high quality control, HQC) 2 µg/ml (PO group) and 100 µg/ml (IV group). All working solutions were prepared in 5 ml volumetric flask and were stored along with the stock solutions at 4°C.

Sample preparation

All plasma samples for TIG determination were thawed at room temperature and vortexed. The volume of 250 µl of turkey plasma transferred into a clean polyethylene 5 ml tube and 25 µl of TIG (for QC samples) and 25 µl of IS (for experimental and QC samples) working standard solution was added. The samples were mixed in a vortex for 10 s (1200 rpm) and 25 µl of 25% formic acid was added. After mixing in a vortex for 10 s (1200 rpm) 750 µl of acetonitrile was added for protein precipitation. Then, after vortexing (3000 rpm, 5 s) and centrifugation (2200 × g for 10 min at 4°C), the supernatant was transferred into a clean polyethylene tube and 1.5 ml of 1,2-dichloroethane was added. After vortexing at 3000 rpm for 30 s, the samples were centrifuged (2200 × g for 10 min at 4°C) and then 150 µl of the superficial layer was transferred through a PTFE Syringe Filter (0.45 µm diameter, ETI, Medlab, Poland) into Total Recovery vials (Waters) and an aliquot was injected for LC-MS/MS analysis.
Method validation

The applied analytical method was fully validated in our laboratory, according to the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) Guidance for Bioanalytical Method Validation.

During the method validation process, specificity, selectivity, linearity, lowest limit of quantification (LLOQ), precision, accuracy, matrix effect, carry-over effect, extraction recovery and stability were evaluated. The limit of detection (LOD) of TIG was established according to the algorithm from MassLynx 4.1 software at the signal to noise ratio above 6:1.

Analysis of individual parameters included: coefficient of variation (CV), standard deviation (SD) and Bias [(mean concentration – nominal concentration) \times 100\%/nominal concentration].

The linearity, accuracy and precision were validated separately for IV and PO administration because of a large difference in concentrations found in plasma between the two administration routes.

Specificity and selectivity

The specificity and selectivity of the method were verified by examining the presence or absence of interference, comparing chromatograms of six individual blank plasma samples from different animals and blank serum spiked with the standard. The absence of interference was accepted when the response was less than 20% of the LOQ for TIG and 5% for the IS.

Linearity

In order to evaluate the linearity, ten calibration standards were studied over a calibration range of 0.01 – 2.0 μg/ml (PO group) and 0.1 – 100 μg/ml (IV group) for TIG in turkey plasma for four days. The least square regression analysis of the data was performed to determine the calibration curve parameters \( y = ax + b \). The calibration curves were constructed by plotting peak area ratios of calibration standards to the IS versus the nominal concentrations of analytes. The back-calculated concentrations of the calibration standards should be within ±15% of the nominal concentration, except for the LLOQ, for which it should be within ±20%.

Precision and accuracy

In order to investigate the intra- and inter-day precision and accuracy, QC samples and LLOQ were determined in six runs during three days. The precision determined at each concentration level should not exceed 15% of the CV, except for the LLOQ, where it should not exceed 20% of the CV. The deviation of the mean concentration from the nominal concentration serves as the measure of accuracy. The mean value should be within 15% of the nominal value except LLOQ, where it should not deviate by more than 20%.

Carry-over effect and LLOQ

The carry-over effect was assessed by injecting a single blank sample following the HQC sample in six replicates and the acceptance criteria was set at ≤20% of LLOQ.

The LLOQ was evaluated by analyzing six replicates of spiked samples in three replications at the concentration of 10 ng/ml with the precision and accuracy within ±20%.

Matrix effect and recovery

The matrix effect was determined by using a calculated ratio of the peak area in the presence of the matrix to the peak area in the absence of the matrix. This test was carried out for the LLOQ and for the HQC in three replicates. The extraction recovery of TIG and IS was carried out at the three QC and the LLOQ in six replicates.

Stability

The stability of TIG in turkey plasma was estimated by an assay of three replicates of QC samples under the following conditions: short-term storage stability after storage at 4°C for 48 h and long-term storage stability after storage at 4°C for 6 days, freeze-thaw stability through three freeze-thaw cycles at -70°C (1, 7 and 30 days) and the postpreparative stability after 24 h and 48 h in the autosampler maintained at 10°C. The QC samples were analyzed in relation to a calibration curve, obtained from freshly spiked calibration standards and the resulting concentrations were compared to the nominal concentrations. The mean concentrations should be within ±15% of the nominal concentration.

Application of the method in a pharmacokinetic study

Ten 4-week-old healthy turkeys, type BIG-6, were obtained from a commercial farm in Stawiguda and
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Table 2. Analytical method validation results of tigecycline (TIG) concentration in turkey plasma.

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>PO administration</th>
<th>IV administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (μg/mL)</td>
<td>Mean SD CV</td>
<td>Mean SD CV</td>
</tr>
<tr>
<td>Linearity (R)</td>
<td>0.998 0.001 0.078</td>
<td>0.995 0.002 0.171</td>
</tr>
<tr>
<td>LOD (μg/mL) for S/N ≥ 6</td>
<td>0.003 0.0001 4.62</td>
<td>0.003 0.001 4.042</td>
</tr>
<tr>
<td>LLOQ (μg/mL) for S/N ≥ 10</td>
<td>0.011 0.001 4.43</td>
<td>0.011 0.001 4.43</td>
</tr>
<tr>
<td>Accuracy (μg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOQ</td>
<td>0.011 0.001 7.667</td>
<td>0.011 0.001 7.667</td>
</tr>
<tr>
<td>LQC</td>
<td>0.028 0.002 7.723</td>
<td>0.105 0.007 5.405</td>
</tr>
<tr>
<td>MQC</td>
<td>0.181 0.013 9.955</td>
<td>34.334 1.162 14.448</td>
</tr>
<tr>
<td>HQC</td>
<td>1.891 0.102 5.434</td>
<td>89.526 9.098 14.14</td>
</tr>
<tr>
<td>Intra-day precision (μg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOQ</td>
<td>0.011 0.001 5.883</td>
<td>0.011 0.001 5.883</td>
</tr>
<tr>
<td>LQC</td>
<td>0.028 0.002 5.716</td>
<td>0.105 0.007 4.695</td>
</tr>
<tr>
<td>MQC</td>
<td>0.181 0.009 5.463</td>
<td>34.334 0.830 2.416</td>
</tr>
<tr>
<td>HQC</td>
<td>1.891 0.038 2.038</td>
<td>16.752 2.350 0.625</td>
</tr>
<tr>
<td>Inter-day precision (μg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOQ</td>
<td>0.011 0.002 18.057</td>
<td>0.011 0.002 18.057</td>
</tr>
<tr>
<td>LQC</td>
<td>0.028 0.002 9.224</td>
<td>0.105 0.014 13.27</td>
</tr>
<tr>
<td>MQC</td>
<td>0.181 0.025 13.989</td>
<td>34.334 2.514 7.321</td>
</tr>
<tr>
<td>HQC</td>
<td>1.891 0.277 14.675</td>
<td>89.526 7.225 8.07</td>
</tr>
<tr>
<td>Total Recovery (%)</td>
<td>TIG</td>
<td>53.19</td>
</tr>
<tr>
<td>IS</td>
<td>30.6</td>
<td>3.505</td>
</tr>
<tr>
<td>Carry-over (%)</td>
<td>TIG (2.0 μg/mL)</td>
<td>0</td>
</tr>
<tr>
<td>IS (0.5 μg/mL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stability (increase/decrease) (%)</td>
<td>Freeze and thaw</td>
<td>Increase/decrease after 144 h in -70°C: 4.474 ± 5.356</td>
</tr>
<tr>
<td>Autosampler</td>
<td>Increase/decrease after 24 h in 10°C: 3.626 ± 4.083</td>
<td></td>
</tr>
<tr>
<td>Working standard</td>
<td>Increase/decrease after 144 h in 4°C: 11.855 ± 3.902</td>
<td></td>
</tr>
<tr>
<td>Matrix effect (%)</td>
<td>Increase/decrease 2.36 ± 7.35</td>
<td></td>
</tr>
</tbody>
</table>

LOD – limit of detection; LLOQ – the lowest limit of quantitation; LQC – low quality control; MQC – medium quality control; HQC – high quality control; IS – internal standard (minocycline); S/N – signal to noise ratio; SD – standard deviation; CV – coefficient of variation.

transported to the animal house of the Faculty of Veterinary Medicine UWM in Olsztyn. The mean body weight of the birds was 1.99±0.24 kg. The vivarium was air-conditioned, the temperature was 22°C and the illumination was automatically controlled (16 h light/8 h dark). The birds had free access to food and water and were observed for one-week acclimatization. Before the experiment, the birds had not been treated with any drugs. After acclimatization, the birds were not fed for 6 h (at night) and thereafter were treated with TIG.

TIG (Tygacil 50 mg, Wyeth Pharmaceutical USA) was dissolved in water for injection and administered at a dose of 10 mg/kg into the left brachial vein (IV group, n=5). The final volume of the drug administered did not exceed 3 ml. The PO group (n=5) received the drug at the same dosage as gavage via a gastric tube directly into the crop. In order to exclude regurgitation, the birds were submitted to observation for 0.5 h after oral administration of TIG. After drug administration, the birds were not fed for 2 h but had access to water. Blood samples (1 ml) were collected into heparinized tubes from the right brachial vein through a venflone canula 26 G (0.6 x 19 mm, HMD Healthcare Ltd. UK) at 0, (0.08 and 0.25 in IV group), 0.5, (0.75 in IV group), 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48 and 72 h after drug administration. Plasma was separated by centrifugation at 1600 × g for 10 min (4°C) and stored at -70°C in an ultrafreezer until analysis. All birds were euthanized by intraperitoneal injection of sodium pentobarbital (Euthasol Vet, Fatto, Poland) at the end of the experiment. The study had been registered and approved by the Local Ethics Commission in Olsztyn (Opinion No. 13/2014).
Results

Method validation

All parameters of the method submitted to validation met the acceptance criteria. The validation results included linearity, LLOQ, LOD, accuracy, precision, total recovery, carry-over effect, stability, matrix effect and selectivity are presented in Table 2.

Specificity and selectivity

The retention time of TIG was about 5.18 min and 6.50 min for IS, and the total running time for one sample was 18 min. No interference peaks from endogenous substances were observed at the retention time of TIG and IS in the chromatograms, which indicates good specificity and selectivity of the method. Examples of ion chromatograms of control samples and LLOQ are given in Fig. 1.

Linearity

The calibration curve for TIG was linear over the concentration range of 0.01 – 2.0 μg/ml (PO group) and 0.1 – 100 μg/ml (IV group). All standard curves from the four validation runs had linear correlation coefficients >0.99 and Bias was <15% for each curve.

Carry-over effect

No significant carry-over effect from the analyte was observed after injecting blank plasma sample immediately after HQC sample at the retention time of TIG and IS.

Precision and accuracy

The testing of the method’s precision and accuracy was accomplished according to three QC points and the LLOQ. The intra- and inter-day precision and accuracy were <15%. All the results achieved during our experiment satisfy the acceptance criteria and prove that the developed method is replicable.

Determination of LLOQ and LOD

LLOQ was determined at an average level of 10 ng/ml and met all acceptance criteria (CV and Bias <15%). The LOD of TIG was determined at the level of 3.014 ng/ml and the signal to noise ratio was over 6:1.

Extraction recovery and matrix effect

The absolute extraction recovery of TIG and IS with the currently reported method was 54% and
Fig. 2. Mean (±SD; n=5) concentration of tigecycline in turkey plasma after single intravenous and oral administration at a dose of 10 mg/kg body weight on semi-logarithmic scale.

31%, respectively. Although TIG recovery was lower than those achieved by Munyeza et al. (2016) and Xie et al. (2014), which were 82.4% and 94.3%, respectively, the method presented in this article is characterized by similarly high sensitivity compared to the methods used in the aforementioned studies. The matrix effect for TIG for turkey plasma was 2.36%, indicating that endogenous components from plasma did not affect the repeatability.

Application of the method: TIG concentration in turkey plasma studies

The mean TIG plasma concentrations versus time profile after single IV and PO administration are shown in Fig. 2. The mean maximum concentration (Cmax) of TIG after IV administration was 53.18±16.81 μg/ml and the time to achieve Cmax (tmax) was 0.08 h, whereas Cmax of TIG after oral administration was 0.102±0.038 μg/ml in tmax of 2 h.

Discussion

Thus far, no-one has described a method for extracting TIG from bird plasma, and all attempts at adapting the available methods to turkey plasma failed to yield satisfactory purity of the analyte or recovery of TIG. Therefore, an effort was undertaken to develop a new, economical and sensitive method for determination of TIG in turkey plasma.
The first step in the analytical method development was to determine the parameters of the mass detector. In the early phase of the experiment, individual ions of the TIG analytical standard were monitored at 586.3 \( m/z \) \( \rightarrow \) 513.3 \( m/z \) ion transition, according to detection parameters described in available literature (Hoffmann et al. 2007, Ji et al. 2007, Ji et al. 2008, Bhattacharya et al. 2014, Xie et al. 2014). However, the sensitivity of the method was very low (\( \geq 400 \) ng/ml), which would not ensure reliable results with respect to pharmacokinetics of TIG administered orally, where the absorption of the drug was much lower than after IV administration. Considering the chemical structure of a TIG molecule, implicating possible double ionization, detection of TIG ions at 293.60 \( m/z \) \( \rightarrow \) 257.10 \( m/z \) and 293.60 \( m/z \) \( \rightarrow \) 265.90 \( m/z \) ion transition was conducted, which considerably enhanced the sensitivity of the method to \( \leq 10 \) ng/ml. The ability of TIG molecule to undergo double ionization was recently confirmed by Munyeza et al. (2016).

The second stage of these studies was development of extraction procedure of TIG from turkey plasma. The available literature contains a description of TIG extraction with SPE method for both human (Conte et al. 2005) and rat plasma (Munyeza et al. 2016). However, in contrast to LLE extraction, SPE method is time- and labour-consuming, because it includes numerous steps needed to perform the entire extraction process. The literature describes the procedure of sample preparation for LC-MS/MS determination of TIG, which involved the addition of acetonitrile (Conte et al. 2005, Muralidharan et al. 2005, Ozcimen et al. 2014), an internal standard dissolved in acetonitrile (Rodvold et al. 2006, Bhattacharya et al. 2014) or trifluoroacetic acid (Hoffmann et al. 2007, Xie et al. 2014) to a sample. However, our test extraction of TIG from turkey plasma with all the above methods was ineffective, which manifested itself by additional, unidentified peaks appearing in chromatograms.

An essential objective of method development was to select a suitable solvent for extraction of TIG and IS. The following extrahents were tested: n-butyl acetate, ethyl acetate, 1,2-dichloroethane, dichloromethane, hexane, diethyl ether, chloroform and methyl tert-butyl ether. Of these compounds, n-butyl acetate and 1,2-dichloroethane were distinguished by a similar recovery of the analyzed substance and IS, which justified their selection for further tests. Modifications of the pH of samples (alkalization or acidification of the environment) were tested, demonstrating that an addition of 25% formic acid raised the recovery of TIG 7-fold after extraction with butyl acetate and 9-fold after extraction with 1,2-dichloroethane. Consequently, the latter solvent was selected as the extrahent in the described method. Moreover, due to the physicochemical properties of butyl acetate, it is more difficult to collect the aqueous phase at the final stage of sample preparation. Subsequently, the volumes of acetonitrile (750 µl) and 1,2-dichloroethane were determined experimentally so as to achieve the highest sensitivity of the method and purity of the analyte.

Taking into consideration that TIG is a derivative of minocycline and all tetracycline group drugs contain a tetracycline nucleus in their structure, an assumption was made that the TIG separation on a chromatographic column would be similar to that of other tetracyclines. An effort was made to adapt the method for determination of oxytetracycline in plasma of broiler chickens developed by our research team and described by Ziółkowski et al. (2016). The chromatographic separation on an Atlantis T3 column (Waters) resulting from TIG gradient elution using 0.1% formic acid in water and 0.1% formic acid in acetonitrile satisfied the expectations regarding the strength of a signal, retention time of TIG and IS as well as the heights of both chromatographic peaks.

The analytical method, elaborated and validated as described above, allowed us to determine, for the first time, the TIG concentration in turkey plasma after single IV and PO administration. The obtained results could be useful in future TIG pharmacokinetic studies.

**Conclusion**

Briefly, this is the first report on determination of TIG in bird plasma using LC-MS/MS and 1,2-dichloroethane as the extrahent. The results obtained indicate that the present method is selective, sensitive, precise, accurate and replicable. An obvious advantage of the method is simple and rapid liquid-liquid extraction, which means that the method is economical and allows rapid and precise assays of TIG concentrations in plasma. The newly developed method ensures such high sensitivity that the TIG recovery at the level of 54% does not deter its application. The conditions of extraction and chromatographic determination of TIG described in this paper could be successfully used for the pharmacokinetic studies of the drug in turkeys.

**Acknowledgements**

This work was supported by the Ministry of Science and Higher Education, National Science Centre Project, No. 2013/09/N/NZ7/0545.
The authors want to thank Dr hab. Tomasz Maślanka and Dr Hubert Ziółkowski for their assistance in this report.

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