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Sensitive spectrofluorimetric methods for determination of ethopabate and amprolium hydrochloride in chicken plasma and their residues in food samples

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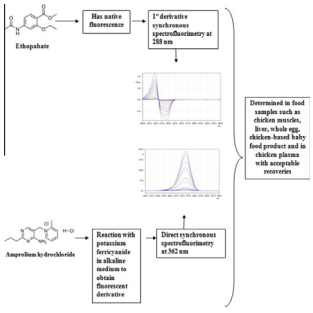
HIGHLIGHTS

- Synchronous spectrofluorimetric methods are used to determine the studied drugs.
- Successful application in different food samples and chicken plasma.
- The proposed methods can be used in food QC without the difficulties of HPLC.
- The proposed work simplifies the extraction and clean-up processes.
- The proposed methods are validated according to ICH guidelines.

GRAPHICAL ABSTRACT



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ABSTRACT

Two sensitive and selective spectrofluorimetric methods are proposed to determine ethopabate (ETH) and amprolium hydrochloride (AMP). First derivative synchronous spectrofluorimetry determines the natively fluorescent ethopabate at 288 nm in presence of amprolium hydrochloride which is a non fluorescent quaternary compound with average recovery 100.54 ± 0.721 over a concentration range of 0.01–0.8 μg/mL. Limits of detection (LOD) and quantification (LOQ) are 0.002 and 0.007 μg/mL, respectively. The second method is direct synchronous spectrofluorimetry for determining amprolium hydrochloride at 362 nm after a reaction with 5% NaOH and 0.08% potassium ferricyanide that is optimized by a two-level factorial design. This method is linear over a concentration range of 0.01–0.65 μg/mL with average recovery 99.4 ± 1.28. Limits of detection (LOD) and quantification (LOQ) are 0.002 and 0.006 μg/mL, respectively. The proposed methods are found to be valid and applicable for the analysis of ETH and AMP in their veterinary formulation. They are successfully applied to determine the studied drugs in chicken plasma and their residues in chicken muscle, liver, egg and chicken-based baby food product with recoveries in the ranges of 95.71–108.73% and 97.36–111.89% and for ETH and AMP, respectively.

Introduction

Ethopabate [methyl 4-acetamido-2-ethoxybenzoate] [1] (Fig. 1) and amprolium hydrochloride [1-(4-amino-2-propylpyrimidin-5-ylmethyl)-2 methylpyridinium chloride hydrochloride] [1] (Fig. 2) are widely used to treat and prevent coccidiosis in chickens.

The US Code of Federal Regulations has established maximum residual limits (MRLs) for ETH to be 0.5 ppm in chicken muscle and 1.5 ppm in chicken liver. It is not allowed to be present in eggs as it must not be given to laying hens. MRLs for AMP are 0.5 ppm in chicken muscles, 1 ppm in chicken liver and 4 ppm in whole egg

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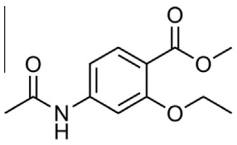

Fig. 1. Structure of ethopabate.

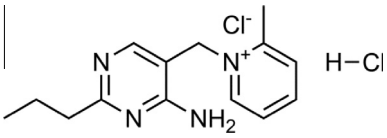

Fig. 2. Structure of amprolium hydrochloride.

[2]. ETH is administered orally in a dose of 125 mg/kg body weight, leading to final plasma concentration of about 16 μg/mL. AMP is administered orally in a dose of 30 mg/kg body weight, leading to final plasma concentration of about 50 μg/mL.

Literature survey revealed that ethopabate and amprolium hydrochloride are official in British Pharmacopoeia [3]. There are many reported methods for the determination of either ETH, AMP together or in combination with other drugs in different matrices such as chicken muscles, chicken plasma, chicken liver and chicken feed. These methods include liquid chromatography coupled with ultraviolet (UV) [4,5] or fluorescence [6–9] detection, and liquid chromatography mass spectrometry (LC–MS) [10–16]. Most of these methods require pre-concentration steps which render them tedious and time-consuming. Different pre-concentration approaches have been used including solid-phase extraction [5], liquid–liquid extraction [16], molecular imprinted polymers [17].

To the best of our knowledge, there is no reported spectrofluorimetric method which determines both drugs simultaneously in bulk powder, veterinary formulation or other matrix.

The aim of the study is to develop simple, rapid, sensitive and selective spectrofluorimetric methods capable of determining ETH and AMP in chicken plasma and their residues in chicken tissues, eggs and chicken-based baby food product, to simplify the extraction and clean-up processes without the need of sophisticated instruments, expensive solvents or large number of samples and to facilitate monitoring of tissue samples for veterinary drug residue violations and hence can avoid potential hazards to human consumers.

Experimental

Apparatus

- Fluorescence is measured on Kontron SFM25 (BIO-TEK Kontron, Switzerland) spectrofluorimeter, equipped with a 150 W xenon lamp and a photomultiplier detector. The spectrofluorimeter is controlled by computer, using SFM25 software, the photomultiplier tube voltage is adjusted at 400 V and the optimum scan speed of 500 nm/s is used. All measurements take place in a standard 1 cm path length quartz cell, where the excitation and emission monochromators are scanned simultaneously with a constant difference $\Delta\lambda = 80$ nm and a response time of 8 s. The slit width of both monochromators is 5 nm and the synchronous spectra are recorded in an excitation scale.
- Jenway® 3510 pH meter calibrated with standard buffers for pH adjustment.
- IKA® T25 Digital Ultra-Turrax® homogenizer with a 7 mm stainless steel generator probe, Germany.
- IKA® Vortex 3 mixer, Germany.
- Kontron® centrifuge PLC series.
- Crest® sonicator, USA.
- Membrane filter (PTFE, 0.45 μm pore size) are used for filtration of samples.
- Thermostatically controlled water bath, Abbota Corp., USA.
- Dragon Med® Calibrated micropipettes.

Software

Minitab® release 14.12.0.

Chemicals and reagents

- Pure standards.

Amprolium hydrochloride and ethopabate are kindly supplied by *PrimaVet* pharmaceutical company, Cairo, Egypt. Their purity is found to be 100.35% and 99.5%, respectively, according to the reported spectrophotometric method [18].

- Standard stock and working solutions.

Stock standard solutions of 100 μg/mL of AMP and ETH are prepared in methanol. Working standard solutions of 10 μg/mL of AMP and ETH are prepared by further dilution with methanol as appropriate.

- Veterinary formulation.

Amprolium & Ethopabate PREMIX 25%® (Batch No. 1203322) is a feed additive labeled to contain 250 g amprolium hydrochloride and 16 g ethopabate per one kilogram, manufactured by Adwia Co., S.A.E., 10th of Ramadan city, Egypt.

- Bi-distilled water is used throughout the whole work and is indicated by "water".
- Methanol, acetonitrile, dimethyl formamide (DMF) are all of spectrofluorimetric analytical grade.
- Sodium hydroxide (Prolabo, Pennsylvania, USA).
- Potassium ferricyanide (Prolabo, Pennsylvania, USA)
- Mixture of 5% sodium hydroxide and 0.08% potassium ferricyanide, as fluorogenic reagent for amprolium hydrochloride, prepared by dissolving 50 g of NaOH and 0.8 g potassium ferricyanide in 1 L of water [8].
- Sodium chloride (Prolabo, Pennsylvania, USA).
- Britton–Robinson buffer (BRB) (pH 2–12) is prepared by mixing different volumes of 0.04 M acetic acid, 0.04 M phosphoric acid, 0.04 M boric acid and 0.2 M sodium hydroxide.
- *Food samples:* Chicken muscle, liver and egg samples are purchased from the local market.
- Chicken with rice baby food (Hero baby®, Switzerland).
- Blood samples are collected from healthy chickens using a cannula inserted into the median cubital vein. Blood samples were centrifuged immediately, and plasma samples were frozen at −20 °C until required for analysis.

Methods

First derivative synchronous spectrofluorimetry for determination of ETH in presence of AMP

Different aliquots equivalent to 0.1–8 μg of ETH are transferred from its working solution into a series of 10 mL volumetric flasks and completed to the volume with water so that the final concentration is in the range 0.01–0.8 μg/mL. Synchronous