Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 586 (2007) 348–358
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Validation of a confirmatory method for the determination of residues of four nitrofurans in egg by liquid chromatography–tandem mass spectrometry with the software InterVal

C. Bock, C. Stachel, P. Gowik *

Federal Office of Consumer Protection and Food Safety (BVL), EU- and National Reference Laboratory (CRL/NRL), Diedersdorfer Weg 1, 12277 Berlin, Germany

Received 31 May 2006; received in revised form 24 October 2006; accepted 1 November 2006
Available online 10 November 2006

Abstract

A method for the detection and determination of nitrofuran derivatives in egg by liquid chromatography–tandem mass spectrometry (LC–MS/MS) was validated with the software InterVal and can be applied for the confirmation of nitrofuran metabolites in fresh or lyophilised eggs. The validation study comprises variations in operator, storage condition, breeding, equipment and duration of sample preparation. A comprehensive overview of the robustness of the method is obtained by analysing eight samples at six concentration levels. First results of short- and medium-term investigations for stability of analytes in solution show that standard solutions of nitrofuran metabolites are stable for at least 1 year when stored at +4 °C in the dark. The decision limit CC_α expressed for the underivatised metabolite is 0.05 μg kg^{-1} for 3-amino-5-methyl-morpholino-2-oxazolidinone, 0.03 μg kg^{-1} for 3-amino-2-oxazolidinone, 0.20 μg kg^{-1} for semicarbazide and 0.22 μg kg^{-1} for 1-amino-hydantoin.

Keywords: Nitrofurans; Matrix-comprehensive; InterVal; Decision limit; Detection capability; Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

1. Introduction

The use of the nitrofurans furazolidone, furaltadone, nitrofurantoin and nitrofurazone as veterinary drugs for food-producing animals is banned by the European Union [1]. The minimum required performance limit (MRPL) for nitrofurans in poultry muscle and shrimps is set at 1 μg kg^{-1} by Commission Decision 2003/181/EC [2] amending Decision 2002/657/EC [3]. But an MRPL for nitrofurans in eggs has not been laid down. In Germany the MRPL of 1 μg kg^{-1} for muscle was translated for egg, predefined in the German National Residue Control Plan.

Because nitrofurans are resorbed, metabolised and distributed very rapidly, only their metabolites are detectable in muscle, liver and kidney as tissue-bound residues [4–8]. From the publication by McCracken et al. [9], it can be assumed that the same is true for nitrofurans and their metabolites in eggs. An evaluation looking for residues of furazolidone and its metabolite 3-amino-2-oxazolidinone (AOZ) showed that after a withdrawal period of 4 days, furazolidone is no longer detectable but its metabolite AOZ still is. In addition the furazolidone concentration in eggs decreases after 55 days at −20 °C by 44%, but the concentration of AOZ residues is stable during this period [9]. So the nitrofuran metabolites act as marker residues for the detection of an illegal use of nitrofurans.

The marker residues identified are AOZ for furazolidone, 3-amino-5-methyl-morpholino-2-oxazolidinone (AMOZ) for furaltadone, 1-amino-hydantoin (AHD) for nitrofurantoin and semicarbazide (SEM) for nitrofurazone. These markers are detected after derivatisation as their nitrophenyl (NP) derivatives by liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10–12] and they are quantified using the isotopically labelled analogues d_4-AOZ and d_5-AMOZ, $C^{13}N^{15}N^{15}$-SEM and $(C^{13})_3$-AHD.

For matrix-comprehensive in-house validation, the databank-oriented software InterVal by quo data GmbH (Dresden, Germany) was used. InterVal allows the automatic calculation of validation parameters like decision limit CC_α and detection capability CC_β, precision, recovery and calibration curves with the respective prediction interval. Validation parameters like the ones named are required according to Commission Decision

* Corresponding author. Tel.: +49 18884122302; fax: +49 18884122955.
E-mail address: crlvetdrug@bvl.bund.de (P. Gowik).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.11.001

2002/657/EC [3]. Additionally InterVal delivers details on the measurement uncertainty and the influence of individual factors, and with this, it provides comprehensive information on the robustness of the method [13]. Trueness and stability have to be determined in further experiments.

The validation results for a method which is to be used for the determination of nitrofuran metabolite residues in egg with the software InterVal are presented and discussed. The underlying method was developed for muscle tissue by RIKILT [14], modified for the analysis of lyophilised or fresh eggs and validated

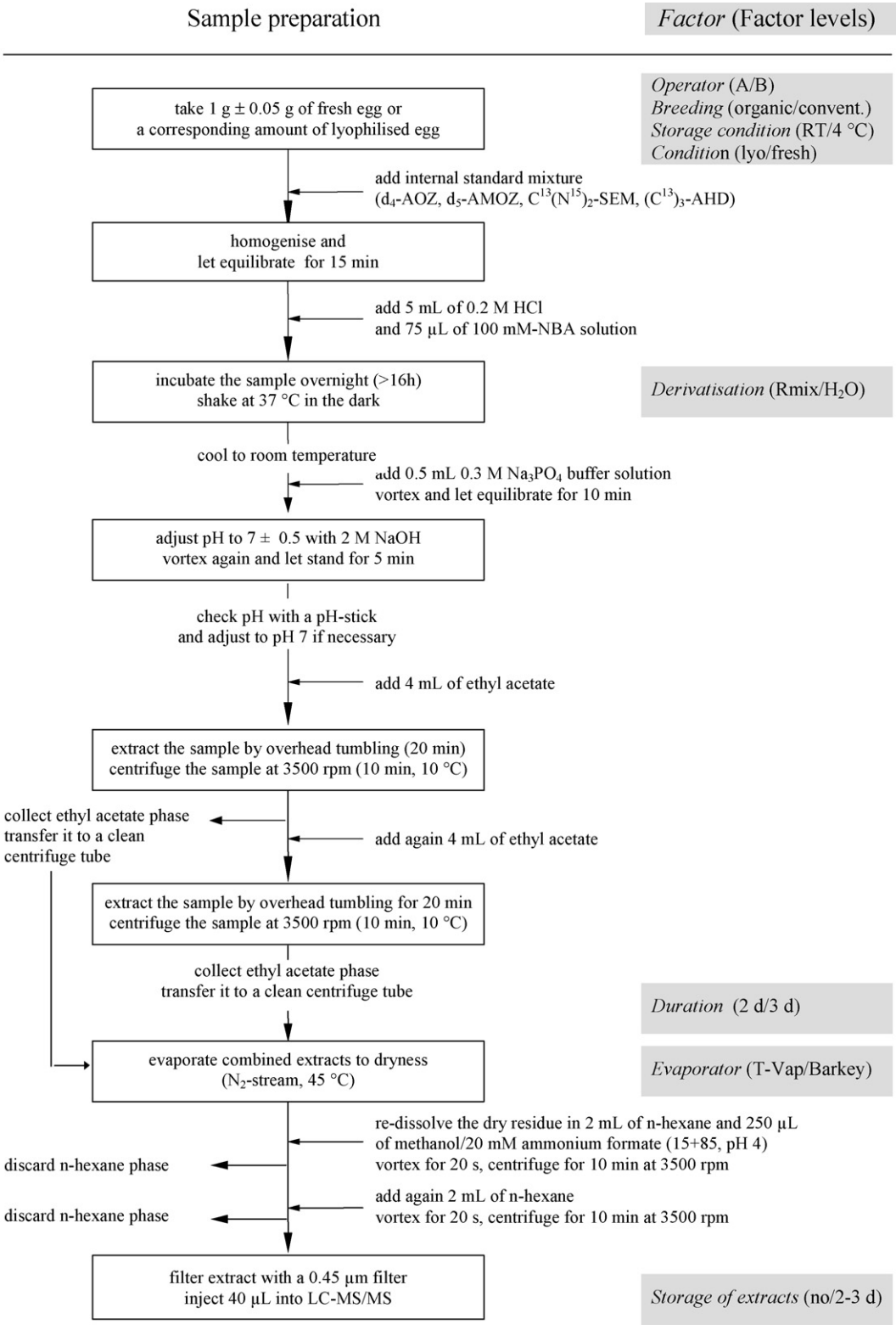

Fig. 1. Sample preparation for the determination of total nitrofuran residues in egg and the validation factors chosen with their factor levels.