GAS CHROMATOGRAPHIC METHODS FOR ANALYSES OF NITROFURAN IN ANIMAL TISSUE AND FATTY ACID METHYL ESTERS IN BIODIESEL

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Methodology

The aim of this research is to develop gas chromatographic mass spectrometry (GC/MS-MS) methods for the analysis of furazolidone in animal tissue which is the most commonly used nitrofuran in animal husbandry. This method will be used to detect metabolites (3-amino-2-oxazolidinone, AOZ) that are stable after long-term storage and can be detected in animal tissue. The preparation of AOZ will be performed with hydrolysis and extraction. The AOZ that is bound to protein will be released in acidic conditions and react with 2-nitrobenzaldehyde to form 3-[(2-nitrobenzyl)methylene]-amino]-2-oxazolidinone (NBAOZ). This preparation helps AOZ to be separated from the matrix and increases the molecular size, which is suitable for separation by gas chromatography techniques under appropriate conditions. It was found that this method provided good linearities (0.0 – 10.0 micrograms per kilogram) with correlation coefficients of 0.995, the lowest detection limit was 1.0 microgram per kilogram, which was higher than the limit of 0.0001 micrograms per kilogram of the standard method (EN14103) for biological diesel B100. The relative standard deviation and relative standard recovery for AOZ in the range of 3.80 – 8.81% and 71.23% met the criteria. The reproducibility of AOZ in animal tissue was found to be 71.5 ± 2.7%.

In the second part of the research, the developed gas chromatographic system was used to analyze fatty acid methyl esters (FAME) in biodiesel B100 and biodiesel-B5 blends. The separation of FAME was performed using a BPX5/BP20 column, which has different elution mechanisms in each column. The LMCS module was used to program the temperature conditions. The developed GCxGC method was found to be suitable for separating and analyzing FAME in biodiesel B100 and B5, and for analyzing FAME from feedstock. This method is superior to the standard infrared method for FAME analysis, which is faster, but may not provide accurate results.
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ABSTRACT

This work describes a method of development for the analysis of furazolidone, the most widely used nitrofuran, in animal tissue using gas chromatography/tandem mass spectrometry (GC/MS-MS). The method monitors the side chain metabolite, 3-amino-2-oxazolidinone (AOZ), which is stable even after long storage and can be detected in the tissues of animal. Sample preparation for AOZ was based on simultaneous hydrolysis and derivatization procedure. AOZ is released from protein-bound residues under an acidic condition, and, subsequent to derivatization with 2-nitrobenzaldehyde (2-NBA), yield 3-[[2-nitrobenzyl]methylene]-amino]-2-oxazolidinone (NBAOZ), respectively. The derivatization step serves to isolate AOZ from the matrix and produces a derivative with a larger molecular mass suitable for GC separation with MS detection. Under optimal condition, a satisfactory validation of data was achieved for linearity, accuracy and precision. An external calibration curve was obtained for a plot between the area ratio with internal standard (diphenylamine) and the concentration of AOZ, ranging from 0.0 – 10.0 µg/kg. The regression coefficient was higher than 0.995. The limit of quantification of the proposed method was found to be 1.0 µg/kg and is within the EU minimum required performance limit (MRPL). Precision in terms of %RSD in repeatability and reproducibility of the GC/MS-MS 3.80 – 8.81 and 18.23%, respectively. Recoveries in animal tissues were ranged from 81% to 92%. The proposed method can be effectively applied to the quantitation of AOZ in animal tissue such as shrimp, chicken and pork.

The second part of this work involves the development of a comprehensive two-dimensional gas chromatography – flame ionization detection (GCxGC-FID). This method is used for analysis of fatty acid methyl esters (FAMEs) in both biodiesel (B100) and biodiesel blend (B5). The orthogonal separation of FAME was based on the boiling point in the 1st dimension and the polarity in the 2nd dimension by using BPX5/BP20 column set, coupled with the LMCS modulator operating in the special temperature programming mode. The developed GCxGC method is successful for characterization and determination of FAME content in B100 and B5, produced from vegetable oils, animal fats and waste cooking oils, with high precision. This method has some advantages over the standard method. The EN 14103 is applicable to only B100 containing FAME C14 to C24, whereas the developed method is applicable for both B100 and B5, and the method is capable of analysis of FAMEs with carbon numbers, C4 to C24. This method was also more robust when compared with the standard infrared spectroscopy (IR) method for B5 analysis. The standard IR method is sensitive to water that may be present in biodiesel, but water does not affect GCxGC analysis.

KEY WORDS: NITROFURAN/ FURAZOLIDONE/ AOZ/ FATTY ACID METHYL ESTER/ BIODIESEL/ COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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