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Net analyte signal standard addition method for simultaneous determination of sulphadiazine and trimethoprim in bovine milk and veterinary medicines

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ABSTRACT

Net analyte signal standard addition method has been used for the simultaneous determination of sulphadiazine and trimethoprim by spectrophotometry in some bovine milk and veterinary medicines. The method combines the advantages of standard addition method with the net analyte signal concept which enables the extraction of information concerning a certain analyte from spectra of multi-component mixtures. This method has some advantages such as the use of a full spectrum realisation, therefore it does not require calibration and prediction step and only a few measurements require for the determination. Cloud point extraction based on the phenomenon of solubilisation used for extraction of sulphadiazine and trimethoprim in bovine milk. It is based on the induction of micellar organised media by using Triton X-100 as an extraction solvent. At the optimum conditions, the norm of NAS vectors increased linearly with concentrations in the range of 1.0–150.0 μmol L^{-1} for both sulphadiazine and trimethoprim. The limits of detection (LOD) for sulphadiazine and trimethoprim were 0.86 and 0.92 μmol L^{-1}, respectively.

1. Introduction

Early detection of contaminants in food requires fast, cost effective and reliable analytical methods that should ideally be accessible to non-skilled users. Sulphonamides and dihydrofolate reductase inhibitors such as trimethoprim belong to a family of broad-spectrum synthetic bacteriostatic antibiotics that are widely used in veterinary medicine as feed additives in most countries (Raich-Montiu, Folch, Compano, Granados, & Prat, 2007). Consequently residues of antibiotics can be present in food products of animal origin, particularly in cow's milk and other dairy products. The importance of testing for antibiotics on farm prior to the collection of milk is well known. Antibiotics used on dairy farms carry the risk of contaminating milk and milk products. Prevention of antibiotic residues in milk has a significant financial benefit and is therefore a key concern for all farmers.

Sulphadiazine belongs to a group of antibiotics called the sulphonamides. Antibiotics are used to treat infections caused by bacteria (Hela, Brandtner, Widek, & Schuh, 2003). In order to grow and multiply in numbers, bacterial cells need to produce genetic material (DNA). To produce DNA they require folic acid (folate). However, bacterial cells cannot take up folic acid supplied in the diet like human cells. Instead, they synthesize it themselves. Sulphadiazine prevents the bacteria for producing folate (Hela et al., 2003). Without folate, the bacteria cannot produce DNA and so are unable to increase in numbers. Sulphadiazine therefore stops the spread of infection. The remaining bacteria are killed by the immune system or eventually die.

Trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine) is a white to cream coloured, odourless and bitter compound. Trimethoprim is an antibiotic used to treat bacterial infections. It works by stopping the growth of bacteria. This antibiotic treats only bacterial infections. It will not work for viral infections (e.g., common cold, flu). Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness. Trimethoprim is more effective when combines with a sulphonamide such as sulphadiazine (Pereira & Cass, 2005). The combination exhibits a greater spectrum of activity against microorganisms that cause infectious disease. The addition of trimethoprim to a sulpha drug forms a potentiated sulpha (Pereira & Cass, 2005). Trimethoprim plus sulphadiazine and trimethoprim plus sulphamethoxazole are the most often-used combinations in veterinary medicine. These are similar in antibiotic function and may be used interchangeably. Trimethoprim-sulpha can kill a variety of bacteria. An additional effect of potentiated sulpha drugs is an ability to kill or suppress certain intracellular parasites, particularly Coccidia spp. and the microorganism responsible for the disease Toxoplasmosis (Pereira & Cass, 2005).

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Several gas chromatographic (Bye & Land, 1977; Lin, Brater, & Benet, 1977), and high-performance liquid chromatographic (HPLC) methods have been described for determination of antibiotics in plasma (Brendel, Meineke, Henne, Zschunke, & De Mey, 1988; Guo, Li, Li, Wang, & Li, 1999; Herraez-Hernandez & Campins-Falco, 1999; Nieder & Jaeger, 1988). The limit of quantitation (LOQ) of HPLC assays ranges between 10 and 50 ng mL^{-1}. Sample preparation is performed by liquid–liquid extraction (Nieder & Jaeger, 1988), solid-phase extraction (Herraez-Hernandez & Campins-Falco, 1999) or column switching (Guo et al., 1999; Herraez-Hernandez & Campins-Falco, 1999). Electroanalytical methods using bismuth-film electrode (Campestrini, Braga, Vieira, & Spinelli, 2010), molecularly imprinted polymer modified carbon paste electrode (Sadeghi, Motaharian, & Moghaddam, 2012), boron-doped diamond electrode (Souza, Braga, Vieira, & Spinelli, 2008) have been applied for determination of sulphadiazine and or sulphamethoxazole in recent years. sulphamethoxazole and trimethoprim have been determined in binary mixtures by kinetic spectrophotometric (Ni & Xiao, 2008), bivariate calibration spectrophotometric (Lopez-Martıinez, Lopez-de-Alba, de-Leon-Rodriguez, & Yepez-Murrieta, 2002), column switching HPLC (Mengelers et al., 1989), LC–MS (Mistri, Jangid, Pudage, Shah, & Shrivastav, 2010) and H-Point Standard Additions Method (Givianrad, Saber-Tehrani, Aberoomand-Azar, & Mohagheghian, 2011). Simultaneous determination of trimethoprim and sulphadiazine reported by LC (Batzias, Botsoglou, Kotsaki-Kovatsi, & Kounenis, 2002) and LC–MS (Croubels, De Baere, & De Backer, 2003; Croubels, Wassink, & De Backer, 2002; Hormazabal, Steffenak, & Yndestad, 1993). To the best of our knowledge, there is no report on determination of sulphadiazine and trimethoprim by using a chemometric approach in bovine milk and veterinary medicines.

In this work, a sensitive, selective, accurate and inexpensive procedure has been described for simultaneous spectrophotometric determination of sulphadiazine and trimethoprim by using the net analyte signal standard addition method (NASSAM) with simultaneous addition of analytes (Hajian & Karamian, 2011). This method is a novel standard addition method based on the net analyte signal (NAS) concept to calculate NAS vectors and attribute them to the analyte concentration using UV–visible spectrophotometry technique.

2. Theory

The net analyte signal was defined (Lorber, 1986) based on spectroscopic methods, as the part of the spectrum of a mixture that is unique for the analyte of interest, i.e., it is orthogonal to the spectra of the interferences. For discussion on the concept of NAS, the conventional notations have been used throughout the following equations. Boldface capital letter used for a matrix, a boldface lower case for a column vector and lightface lower case italic for a scalar. The superscript "+" denotes the pseudo-inverse of a non-square matrix. The digitized spectrum is referred to a spectrum vector or simply as a vector, while a spectrum vector of a pure component is called a component vector.

Consider a synthetic mixture containing sulphadiazine (SFD) and trimethoprim (TMP) in concentration of 10.0 μmol L^{-1} for each analyte. The simultaneous determination of two analytes by NASSAM requires having spectrum vector of the mixture. The known amounts of SFD and TMP are successively added to the sample solution. The absorbances are expressed by the following equations:

$$A_0 = \varepsilon_{SFD} C^0_{SFD} + \varepsilon_{TMP} C^0_{TMP} \quad (1)$$

$$A_1 = A_0 + \varepsilon_{SFD} C_{SFD,s_1} + \varepsilon_{TMP} C_{TMP,s_1} \quad (2)$$

$$A_i = A_{i-1} + \varepsilon_{SFD} C_{SFD,s_i} + \varepsilon_{TMP} C_{TMP,s_i} \quad (3)$$

$$A_n = A_{n-1} + \varepsilon_{SFD} C_{SFD,s_n} + \varepsilon_{TMP} C_{TMP,s_n} \quad (4)$$

where "A_0" and "A_i" are the absorbances for the synthetic mixtures before and after of standard additions. C^0_{SFD}, C^0_{TMP} and C_{SFD,s_i}, C_{TMP,s_i} are the initial added concentrations of sulphadiazine and trimethoprim to the sample in the ith step with the molar absorptivities of ε_{SFD} and ε_{TMP} respectively. The vectors of net analyte signals for sulphadiazine and trimethoprim after each standard addition, $NAS_{SFD,i}$ and $NAS_{TMP,i}$ can be calculated by the following equations:

$$NAS_{SFD_i} = (I - R^+R)A_i \quad (5)$$

$$NAS_{TMP_i} = (I - S^+S)A_i \quad (6)$$

where "I" is an identical matrix, "R" and "S" are the matrixes of absorbances at different concentrations of interferences. By definition, it is always possible to split up the spectrum of sample "A_i" into two distinct parts: NAS_{SFD} which is orthogonal to the spectra of the interference (TMP) and the other part is R^+RA_i. NAS_{SFD} is orthogonal to the spectra of the interference, reflecting the part of the spectrum which is only depending on the concentration of sulphadiazine. Similar expressions can be used for trimethoprim. Therefore the orthogonal vectors of SFD and TMP which known as NAS_{SFD} and NAS_{TMP} can be used for quantification of the analytes. Fig. 1 shows the geometrical presentation of the vectors: analyte, interferent, mixtures, T^+TA_i and NAS_{SFD}. The shape of NAS_{SULFA} only depends on the presence of interferences in the mixture, not on their specific concentrations and only addition or deletion of components can change it. In the following, it is assumed that spectra of samples without the analyte are available and are remained constant during the determination. In binary and/or ternary mixtures when the interferences are known, the NAS can be calculated for each analyte. Norm of the NAS vector can be used to construct a univariate calibration model, where this parameter is plotted against the analyte concentration and a linear relationship is observed. In the case of matrix effect, standard addition plots can be constructed.

3. Materials and methods

3.1. Initial investigation

To demonstrate the analytical applicability of the proposed method for the analysis of binary mixtures, two pure spectra of

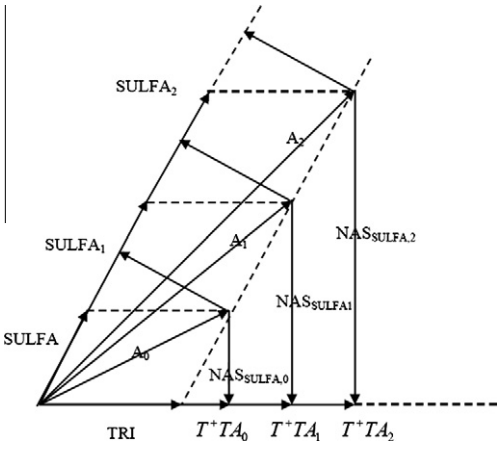

Fig. 1. Representation of vector space for analyte (sulphadiazine) and interferents (trimethoprim) in two dimensional space. NAS vector NAS_{SFD} is different from SFD vector in direction and length.