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Evaluation of matrix effect in isotope dilution mass spectrometry based on quantitative analysis of chloramphenicol residues in milk powder

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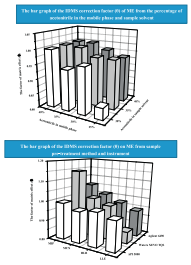
HIGHLIGHTS

- We develop a strategy to evaluate matrix effect and its impact on the IDMS results.
- Matrix effect and IDMS correction factor from different conditions are evaluated.
- Ion suppression effect is observed in LLE and HLB pre-treated sample solutions.
- Ion enhancement effect is found in MCX pre-treated sample solution.
- IDMS correction factor in HLB and MCX solutions in three instruments is close to 1

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, we developed a comprehensive strategy to evaluate matrix effect (ME) and its impact on the results of isotope dilution mass spectrometry (IDMS) in analysis of chloramphenicol (CAP) residues in milk powder. Stable isotope-labeled internal standards do not always compensate ME, which brings the variation of the ratio (the peak area of analyte/the peak area of isotope). In our investigation, impact factors of this variation were studied in the extraction solution of milk powder using three mass spectrometers coupled with different ion source designs, and deuterium-labeled chloramphenicol (D5-CAP) was used as the internal standard. ME from mobile phases, sample solvents, pre-treatment methods, sample origins and instruments was evaluated, and its impact on the results of IDMS was assessed using the IDMS correction factor (θ). Our data showed that the impact of ME of mobile phase on the correction factor was significantly greater than that of sample solvent. Significant ion suppression and enhancement effects were observed in different pre-treated sample solutions. The IDMS correction factor in liquid–liquid extraction (LLE) and molecular imprinted polymer (MIP) extract with different instruments was greater or less 1.0, and the IDMS correction factor in hydrophilic lipophilic balance (HLB) and mix-mode cation exchange (MCX) extract with different instruments was all close to 1.0. To the instrument coupled with different ion source design, the impact of ME on IDMS quantitative results was significantly different, exhibiting a large deviation of 11.5%. Taken together, appropriate chromatographic conditions, pre-treatment methods and instruments were crucial to overcome ME and obtain reliable results, when IDMS methods were used in the quantitative analysis of trace target in complex sample matrix.

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1. Introduction

Over the past few decades, high performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has been widely used in food security, medical science, pharmaceutical industry and biochemistry [1–3]. LC–MS/MS can increase the selectivity, sensitivity and throughput toward the identification and quantification of compounds in samples. However, the ionization process in the mass spectrometer can be interfered by molecules derived from the sample matrix that are co-eluted with the compounds of interest [4]. Previous studies have shown that co-eluting compounds can negatively affect (ion suppression) or positively affect (ion enhancement) the analyte signal in LC–MS/MS analyses. This observation is commonly referred to as "matrix effect (ME)", which is responsible for poor and unreliable data in a quantitative assay. ME can greatly affect the reproducibility, linearity and accuracy of the method, eventually leading to erroneous quantitation. Kebarle and Tang first observed the ME in ESI, where the analyte response is decreased in the presence of other organic compounds [5]. Several other investigations have described the mechanism of ME, methodologies to detect and sample preparation procedures to minimize such effect [6–8]. The primary ME associated with LC–MS/MS methods includes the ion suppression and enhancement. They are caused by the presence of less volatile matrix components (salts, ion pairing agents, endogenous compounds, drugs, metabolites and proteins), which in turn affect the amount of charged ion in the gas phase. Different mechanisms have been proposed to interpret this observation although the actual mechanism remains unclear. The possible explanation can be as follows. Matrix compounds compete with analyte for the limited charge on the droplet surface, and the interfering compounds increase the droplet's viscosity and surface tension, resulting in the affected ionization of analyte and the decreased solvent evaporation rate. It has been also suggested that non-volatile materials in the matrix may decrease the droplet formation rate through the co-precipitation of analyte; therefore, they can prevent droplets from reaching the critical radius required for the emission of gas phase ions [9]. The change in competition between matrix ions and analytes ions inside the ionization source causes the ion suppression or enhancement, eventually affecting the reproducibility and accuracy of the results. Therefore, in order to eliminate or minimize ME, an evaluation should be performed during the method development [10–12]. Buhrman et al. [13] reported the ion suppression effect of co-eluting compounds in quantitative determination of 27,417 SR (2-[*N*-(2-dimethylaminoethyl)-*N*-(3pyridinylmethyl) amino]-4-(2,3,6 tri-isopropylphenyl) thiazole) in human plasma using electrospray HPLC–MS/MS. Others [14–16] studied the ME and its serious interferences on sensitivity, linearity, accuracy and so on. Trufelli et al. [17] summarized two major approaches to estimate ME as follows: post-extraction addition and post-column infusion. In post-extraction addition, the response of analyte in a standard solution is compared with that of a post-extraction spiked sample at the same concentration. The ME in terms of matrix factor (MF) can be calculated using the peak areas. The difference in mass spectrometry response signal suggests the existence of either ion suppression or enhancement. When MF equals to 1, it indicates the absence of any ME, whereas values of <1 or >1 suggest a suppression or an enhancement of the ionization process, respectively [13,18]. Qualitative evaluation of ME has been achieved by using post-column infusion method [4,19] to identify the retention time zones in a chromatographic plot. Following the injection of an extract from a blank sample, the signal of a constantly infused analyte is monitored and compared with the signal without an extract injection. From the spectrogram, it is shown that the response at the retention time zone is reduced or enhanced.

In order to develop a sensitive and reliable analytical method, a great number of methods have been introduced to overcome or eliminate the interference of ME as far as possible during quantitative analytical LC-MS/MS measurements. Some of them [23–25] have been described, including the application of injecting smaller volumes, diluting the sample, optimizing the sample preparation [20–22], adjusting chromatographic and mass spectrometric conditions. However, the main strategy is based on the use of standard addition method, external matrix-matched standards and structural analogue internal standards. Internal standard methods usually use a structural analogue internal standard. However, the ionization of the analogue internal standard and analytes may be differently affected by the matrix [26,27]. Apart from these methods, the other effective approach to compensate ME could be introducing appropriate stable isotope-labeled isotope into matrix due to its almost identical behavior to the target analyte in sample treatment, chromatography, as well as in ionization. The behavior of targets is consistent with that of a well-balanced isotope, consequently minimizing the influences of sample preparation methods and instruments. This isotope dilution mass spectrometry (IDMS) approach possesses advantages of traditional internal standard method. IDMS provides results with a higher metrological quality, such as a wide dynamic range, high sensitivity and accuracy, than those provided by standard additions or external calibration [28,29].

Although IDMS technique can effectively compensate the ME in some situations, matrix, instrumentation and other factors may still affect the peak area ratio of analyte to isotope, consequently leading to a biased result. Lindergardh et al. [30] noticed that remained salts from buffers used in the solid phase extraction suppress the signals of piperaquine and its deuterated internal standard (D6-piperaquine) differently. When the amount of triethylamine in the sample reaches 0.05%, the ion suppression at the peak maxima of the analytes is decreased by as much as 50% from piperaquine to D6-piperaquine, leading to a lower analyte-to-IS ratio and an incorrect quantification. Jian et al. [31] showed that a potential quantitation bias for metabolites can be caused by using the stable isotope-labeled (STIL) parent drug as the internal standard in an LC–MS/MS assay. Ion suppression of the parent drug to its co-eluting STIL parent drug results in the overestimation of metabolite concentrations in the incurred samples, giving rise to misleading information. Taking into account the possible differences of MS response and ME between the target and its polysubstituted isotope markers, González-Antuña et al. [32] minimized the ME during the trace determination of β-agonists in complex matrices using singly 13C-labeled analogue labeling and isotope pattern deconvolution (IPD). Using this approach, they obtained accurate and precise results in the simultaneous quantification of β_2-agonists in human urine and bovine liver, even at the sub ng $g^{-1}$and particularly in spite of the previously reported matrix effects. IDMS has been recognized as a primary measurement approach by the Consultative Committee for Amount of Substance (CCQM) [33]. Therefore, it is extremely necessary to clarify the ME-induced variation of peak area ratios of analyte to its stable isotope-labeled internal standard. However, the effect of chromatography and mass spectrometer conditions, instruments and sample pre-treatment methods on the ME of analyte and its stable isotope internal standard in IDMS-based approaches has been scarcely reported.

The European Union has banned and controlled chloramphenicol (CAP) in animals and their products, and the LOD of CAP in real samples is at ppb-concentration levels. IDMS method has been widely used in trace analysis of CAP in animal-source food. In this work, taking CAP as target analytes and D5-CAP as an isotopically labeled internal standard, we investigated the ME of IDMS-based quantitative method in drug residue analysis from milk powder matrix. The ME of CAP and D5-CAP from mobile phases, sample