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# Chemometrics-enhanced full scan mode of liquid chromatography–mass spectrometry for the simultaneous determination of six co-eluted sulfonylurea-type oral antidiabetic agents in complex samples

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ABSTRACT

In this work, a rapid chemometrics-enhanced liquid chromatography–single stage mass spectrometry (LC–MS) was developed for the simultaneous green determination of six co-eluted sulfonylurea-type oral antidiabetic agents (SOADs) in health teas and human plasma samples. Shortening the chromatographic separation time and reducing the cost per analysis were achieved *via* using full scan mode of LC–MS under simple isocratic elution condition followed by an environment-friendly "mathematical separation" strategy. The problem of the complete separation of target analytes from each other and/or from the uncalibrated interferences in complex matrices was resolved by alternating trilinear decomposition (ATLD) method as *a posteriori*. Satisfactory qualitative and quantitative results were obtained even in the existence of unknown interferences and the "second-order advantage" was fully exploited. The average spiked recoveries for all target analytes were between 81.6% and 110.1% with standard deviations less than 7.7%. It was demonstrated that the proposed strategy could be promisingly used for green resolution and determination of co-eluted multi-analytes of interest in complex samples while avoiding elaborate sample pretreatment steps and complicated experimental conditions as well as more sophisticated high-cost instrumentations.



## 1. Introduction

Type II diabetes is a metabolic disorder characterized by hyperglycemia due to cellular resistance to insulin, combined with insufficient pancreatic secretion of insulin. According to a recent research, the worldwide prevalence of diabetes in 2010 among adults was estimated to be ~285 million (6.4% of the world's population), while this number will increase to 7.7%, resulting in 439 million diabetes by 2030 [1]. Moreover, a report released by the World Health Organization (WHO) indicated about 1.5 million people died from diabetes in 2012. And not only that, WHO predicted that diabetes will be the 7th most important cause of death in 2030 [2]. Glipizide, tolbutamide, gliclazide, glibenclamide, glimepiride and gliquidone belong to sulfonylurea-type oral antidiabetic agents (SOADs) which can increase insulin release from pancreatic beta cells and have been widely used over the past six decades for treating type II diabetes. Common adverse side-effects caused by the abuse of these drugs include life-threatening hypoglycemia, obesity, gastrointestinal upset, skin allergy and liver or kidney damage [3–6].

Nowadays, many patients choose not only the oral antidiabetic agents but also health products to control blood glucose levels with minimal side-effects. However, in order to cater for consumer preferences and pursue commercial profits, some dishonest manufacturers illegally adulterate health products with synthetic antidiabetic drugs without labeling, which bring a lot of potential side-effects and make the public health at risk. Beyond that, it is reported that SOADs are also illegally used as a stopper in racehorses by reducing the blood glucose levels [7,8]. Thus, rapid, reliable, sensitive, and accurate methods are required for screening and determination of synthetic SOADs in "antidiabetic" health products and human plasma samples to avoid the harmful effects of these synthetic drugs.

There are various analytical methods that have been reported to screen and quantify one or more SOADs in health products and clinical biological samples [3–13]. Most of them are based on high-performance liquid chromatography (HPLC) method with some nonselective detectors such as ultraviolet, diode array, or fluorescence detection [10–13]. These methods, however, may lack specificity and thus usually need multiple clean-up steps or long gradient elution programs to separate target analytes from each other and/or from the uncalibrated interferences in complex samples [7,8]. But even if doing that, sometimes it is still difficult to realize complete chromatographic separation of the analyte(s) of

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interest from the complex matrices because of the existence of overlapped or embedded peaks, let alone these clean-up steps and long gradient elution programs often take up the majority of the total analysis time and require significant amounts of toxic organic solvents and chemicals. Consequently, mass spectrometers including both single-stage mass spectrometers (MS) and tandem mass spectrometers (MS/MS), as a selective detector for LC system, have naturally become a better choice for improving the analytical specificity [3–9].

In this context, LC–MS(/MS) has been widely applied to the analysis of target analyte(s) in complex samples ranging from small molecules [9,14] to large protein mixtures [15]. But in practical applications, LC–MS also suffers from some drawbacks. The fundamental problems related to the LC–MS analysis are its peak and/or baseline drifts, spectral backgrounds, noises, low signal-to-noise (S/N) ratio and co-elution (overlapped and/or embedded peaks) [8,9,14,16]. Apart from these, another main problem in LC–MS analysis derives from the low content of target analytes. The trace concentration usually prevents the use of commercial data-analysis tools to eliminate the strong background signals and large baseline variations [17]. As a result, on one hand, the similarity of spectral backgrounds with the mass spectra of target analytes in the presence of noise and baseline mass channels averts identifying the latter. On the other hand, the presence of overlapped peaks of target analytes themselves in LC–MS analysis is a serious factor that hinders a thorough quantitative determination of them. All these facts have compelled researchers to use more advanced LC–MS/MS instead of LC–MS [9,14,16]. However, as a Chinese proverb goes, gold can't be absolutely pure. In addition to the high-cost sophisticated apparatus and specialized operation skills [18], LC–MS/MS typically requires time-consuming optimization procedures for various tandem mass spectrometric parameters such as fragment voltage, collision energy and precursor-product ions.

Fortunately, with the rapid development of chemometrics, the barriers to LC–MS mentioned above may be readily solved in a smart and green way. Among chemometric methods, second-order calibration methods based on three-way data (e.g., LC-DAD, LC–MS or GC-MS data) allow the determination of analyte(s) of interest in complex samples with unresolved peaks and uncalibrated interferences. This flexible strategy is known as "mathematical separation", born with a distinctive property called "second-order advantage" [19], which avoids the requirement of interferences removal, with the concomitant saving of experimental work, toxic organic solvents and analysis time and cost. In this sense, "mathematical separation" based on second-order calibration nicely caters for the goal of handling the problems derived from the instability of LC–MS system, i.e., peak/baseline shifts, spectral backgrounds, noises, low S/N ratio, or even the co-elution problems. More specifically, for the case of LC–MS operating in full scan mode, the response of one sample is arranged as a two-way data matrix where each column corresponds to an $m/z$ ratio and each row corresponds to an elution time. Different chromatographic runs would then generate a three-way data array in which the sample number serves as the third dimension. This three-way data array has an inherent mathematical structure called trilinearity and it can be uniquely decomposed by the second-order calibration methods. In such a way, the profiles for each underlying factors including co-eluted analyte(s) of interest, interfering compounds, spectral backgrounds, noises and even peak/baseline shifts can be mathematically liberated from the mixed response signals.

A variety of second-order calibration methods capable of achieving "second-order advantage" can be employed to analyze the three-way data generated by hyphenated chromatographic techniques. These methods belong to three main groups: (i) direct solution group, including generalized rank annihilation method (GRAM) [20] and direct trilinear decomposition (DTLD) [21]; (ii) iterative solution group, such as parallel factor analysis-alternating least squares (PARAFAC-ALS) [22], alternating trilinear decomposition (ATLD) [23], multivariate curve resolution-alternating least squares (MCR-ALS) [24], multi-way PLS (N-PLS) and unfolded partial least squares (U-PLS), both combined with residual bilinearization (RBL) [25–27]; (iii) least-squares group, e.g., bilinear least squares coupled with residual bilinearization (BLLS/RBL) [28]. However, it should be noted that none of them is versatile because each method has its inherent advantages and deficiencies, and the application objects of these methods are not the same. Recently, some reviews and tutorials have been published to discuss the properties of second- or higher-order calibration methods in terms of theories and applications in detail [29–36].

To the best of our knowledge, only a few works [37–42] have been conducted by utilizing second-order calibration methods in combination with LC–MS. What is more, we have not yet found any chemometrics-based methods for the determination of SOADs in complex samples. Therefore, in this work, a smart strategy that couples three-way LC–MS data with "mathematical separation" based on ATLD method was developed for the resolution and determination of six co-eluted SOADs in health teas and human plasma samples. Through complementary combination of the two methods, one may avoid using the expensive LC–MS/MS method to analyze this type of drugs. Moreover, it is proved that by doing so, the sensitivity and detection limit of the analysis will be improved. The aim of this study is to use simple sample clean-up steps and experimental conditions, reduce chromatographic run time and toxic organic solvent consumption, decrease the cost per analysis and consequently contribute anything beneficial to the green analysis of SOADs.

## 2. Theory

### 2.1. Trilinear component model for second-order calibration

For the case of LC–MS operating in full scan mode, one given sample can generate a two-way response matrix containing the mass spectra at all elution times ($i = 1, 2, ..., I$) in its rows, and the chromatograms at all spectral $m/z$ channels ($j = 1, 2, ..., J$) in its columns. As shown in Fig. 1, when $K$ such matrices, generated by both calibration and prediction samples, were stacked along the sample dimension, then a three-way LC–MS data array $\underline{\mathbf{X}}_{I \times J \times K}$ could be obtained. Supposed that no obvious elution time/baseline shifts and shape deformations occurred for the peaks from run to run, this three-way data array would have an inherent mathematical structure named trilinearity which can be expressed as:

$$x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk}, \text{for } i = 1, 2, ..., I; j = 1, 2, ..., J; k = 1, 2, ..., K \quad (1)$$

where, $x_{ijk}$, the element of $\underline{\mathbf{X}}$, is the ion intensity for the $k^{\text{th}}$ sample at the $i^{\text{th}}$ elution time and the $j^{\text{th}}$ $m/z$ channel; $a_{in}$, $b_{jn}$ and $c_{kn}$ are the elements of resolved normalized chromatograms matrix $\mathbf{A}_{I \times N}$, normalized mass spectra matrix $\mathbf{B}_{J \times N}$ and relative concentrations matrix $\mathbf{C}_{K \times N}$, respectively; $e_{ijk}$ is the element of the three-way residual data array $\underline{\mathbf{E}}_{I \times J \times K}$. $N$ represents the total number of underlying factors generating the mixed response signals, including co-eluted analyte(s) of interest, interfering compounds, spectral backgrounds, noises and even peak/baseline shifts.

The basis of "second-order advantage" is that the measured three-way data array, $\underline{\mathbf{X}}$, can be uniquely decomposed into three underlying matrices which can provide access to pure qualitative profiles ($\mathbf{A}$ and $\mathbf{B}$) and quantitative information ($\mathbf{C}$) of individual component of interest in any unknown samples containing potential interferences. Identification of the chemical constituents under study is achieved with the assistance of the two retrieved qualitative profiles: chromatograms and mass spectra, and comparing them with those for a pure solution of the analyte of interest. The final concentration of each target analyte is found by interpolation of the relative concentrations in the prediction samples into the pseudo-univariate calibration curve established from regressing the relative concentrations in the calibration samples against