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Using second-order calibration method based on trilinear decomposition algorithms coupled with high performance liquid chromatography with diode array detector for determination of quinolones in honey samples

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ABSTRACT

A novel strategy that combines the second-order calibration method based on the trilinear decomposition algorithms with high performance liquid chromatography with diode array detector (HPLC-DAD) was developed to mathematically separate the overlapped peaks and to quantify quinolones in honey samples. The HPLC-DAD data were obtained within a short time in isocratic mode. The developed method could be applied to determine 12 quinolones at the same time even in the presence of uncalibrated interfering components in complex background. To access the performance of the proposed strategy for the determination of quinolones in honey samples, the figures of merit were employed. The limits of quantitation for all analytes were within the range 1.2–56.7 μg kg^{-1}. The work presented in this paper illustrated the suitability and interesting potential of combining second-order calibration method with second-order analytical instrument for multi-residue analysis in honey samples.

1. Introduction

Determination of quinolones in food samples has been significantly and progressively increased in recent years because of the growing problem of microbial resistance and having, therefore, important consequences for public health. Particularly, residues of these drugs in food are important concerns. To reduce the risk in human health associated with the consumption of residues of these compounds, many countries have established a series of the maximum residue limits (MRLs) for quinolone residues in foods. Traditional techniques used for the determination of residues of these compounds are mainly based on high performance liquid chromatography with diode array detector (HPLC-DAD) [1–5], mass spectrum (LC–MS) [5–10], fluorescence detector (LC-FD) [5,11–14], etc. However, there is a common problem that time and organic solvents are consumed for these traditional methods in the case of multi-samples analysis. Usually, a long time or much more complex chromatography condition is required by these traditional methods for the simultaneous determination of multi-components. More recently, it was reported that there is a need for simplifying the complicated, time-consuming multi-sample pretreatment and the analysis procedure [15]. It is worth noting that co-elution or interfering compounds maybe also exist, which will, of course, complicate the analysis procedure and result in unacceptable results in terms of neither qualitative results nor quantitative results. Fortunately, these overlapped peaks can be resolved well with the aid of chemometric methods. The main advantages of combining chromatography method with second-order calibration include: (1) the chromatography condition can be simplified. Usually, only an isocratic mode is needed. (2) The efficiency of analysis procedure can be significantly improved. Generally, less time is needed with the aid of chemometric methods because the overlapped peaks can be resolved, suggesting less attention would be paid on the scope of isolating chromatographic peaks of each components. There are also several works presented for the determination of qua in honey samples [16–18]. In this paper, unlike the traditional chromatography methods, the work presented here, is aimed at employing chemometric methods to quantify analytes even in the presence of overlapping peaks to make the analytical procedure much more efficient and simple.

Nevertheless, whether the analytes can be successfully quantified in the presence of interferents depends on the type of instruments and also on the chemometric methodology. According to the published papers [19–21], data generated from instruments for a single sample can be classified as zero-, first-, second- and higher-order tensors, and the corresponding chemometric

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methods are named as the zero-, first-, second- and higher-order calibration methods, respectively. Among these calibration methods, first-order calibration methods such as partial least squares (PLS), multivariate linear regression (MLR) cannot give reasonable results for the samples containing uncalibrated interfering analytes. Methods used for the samples containing uncalibrated interfering analytes can be classified into two categories: (i) multivariate curve resolution methods which are employed to interpret a single sample data represented by iterative target transformation factor analysis (ITTFA) [22], window factor analysis (WFA) [23], heuristic evolving latent projections (HELP) [24,25], multivariate curve resolution-alternating least squares (MCR-ALS) [26], etc. and (ii) methods which are utilized to deal with multi-samples. The latter type includes (a) methods based on generalized eigenvalue problem such as generalized rank annihilation method (GRAM) [27,28], direct trilinear decomposition (DTLD) [29], (b) methods based on alternatively optimizing trilinear target functions such as parallel factor analysis (PARAFAC) [30,31], alternating trilinear decomposition (ATLD) [32], and (c) methods based on unfold strategy, for example, unfold partial least squares combined with residual bilinear least squares (U-PLS/RBL) [33] which unfolds each sample into a vector, and multivariate curve resolution-alternating least squares (MCR-ALS) [26], etc.

Multivariate curve resolution methods, under certain proper constrains, also have the second-order advantage that can quantify analytes even in the presence of uncalibrated interfering signals for instance, there is a need of selective and zero regions for HELP. It is worthy to point out that, like MCR-ALS, these single-sample methods can be also extended to resolve multi-sample data set. In contrast to second-order calibration methods based on trilinear decomposition algorithms such as GRAM and PARAFAC which require the three-way data have a low-rank trilinear structure, a main advantage of these multivariate curve resolution methods is that only low-rank bilinear structure is needed for augmented data; that is to say, these methods have the additional ability of handling with the data set in the presence of time shift problem. But all of these methods may face the potential problem of rotation ambiguities; in other words, it is probably that infinite numbers of solutions will be obtained, particularly in case of analyzing components without selective information.

A superiority of trilinear decomposition methods over bilinear decomposition methods specializes in the fact that the solutions from former are always unique in the profiles, which avoid the rotation ambiguities and can fully obtain the second-order advantage. Several reviews [19,34,35] have demonstrated the useful potential of applying trilinear decomposition methods coupled with modern analytical instruments to quantify analytes of interest in food samples. In this paper, we focus our attention on the quality control of honey production. Honey samples have been shown a complex background. Traditionally, a lot of trivial pretreatments are necessary to separate the interfering compounds from analytes of interest, for example, solid phase extraction [15]. Moreover, it may take a long time and/or complex mobile phase to isolate each of the analytes to provide accurate results. The present work has firstly demonstrated that 12 quinolones, Cirprofloxacin (CIP), Danofloxacin (DAN), Difloxacin (DIF), Enoxacin (ENO), Enrofloxacin (ENR), Fleroxacin (FLE), Lomefloxacin (LOM), Marbofloxacin (MAR), Ofloxacin (OFL), Orbfloxacin (ORB), Pefloxacin (PEF), and Sarafloxacin (SAR) in honey samples can be quantified even in the presence of uncalibrated interfering components using high performance liquid chromatography diode array detector with the aid of second-order calibrarion method based on trilinear decomposition algorithms. Compared with the traditional chromatographic methods, this new method has the advantages of less time-consumption and less organic solvent consumption as well as the advantage of making the analysis procedure more effective and simple.

2. Experiment

2.1. Chemicals and solutions

Quinolones used in this paper are shown in Fig. 1 and were purchased from Sigma–Aldrich. Methyl alcohol (TEDIA Company, USA) and cyclohexane were of HPLC-grade. Formic acid was purchased from Adamas Reagent Company (99%). All of the reagents were of analytical reagent grade.

Stock solutions ($1\,mg\,ml^{-1}$) of quinolones were prepared by dissolving the compounds in aqueous methanol (ultrapure water:methanol = 20:80, v/v). All solutions were stored in at 4 °C and were stable over 3 months.

Mobile phase (pH 2.5) consisted of methanol and 1% aqueous formic acid (methanol:1% aqueous formic acid = 29:71, v/v). The eluent was filtered prior to the usage at a flow rate of $1\,ml\,min^{-1}$. The injection volume was 10 μl. Working standard solutions were freshly prepared by diluting stock solutions with mobile phase.

2.2. Apparatus and instruments

A 3K30 ultracentrifuge with cooling system (Sigma, St. Louis, MO, USA) and a rotary evaporator system with heating pool were used.

The chromatography system was of a LC-20AT (Shimadzu, Japan). Chromatographic separation was achieved on a WondaSil C18 column (5 μm, 150 mm × 4.6 mm). Detection was performed with a diode array detector. Spectra were measured at a range of 200–430 nm, monitoring wavelength at 280 nm and 350 nm. A Dell computer, using windows XP software was used.

2.3. Samples

2.3.1. Honey samples

Honey samples were purchased from local market. A 10 g amount of sample was transferred to a 50 ml centrifuge, 30 ml of acetonitrile was added and the mixture was sonicated for 15 min. The tube was subsequently stopped and centrifuged for 10 min at 7000 rpm. The extracts of honey sample were mixed and were centrifuged again. Finally, extracts were degreased using 40 ml hexane and were evaporated to dryness at 50 °C and then diluted to 1 ml with mobile phase. The resulting solution was injected into the HPLC system.

2.3.2. Calibration samples and validation samples

Concentrations of 12 quinolones in the calibration samples ranged around from 1 to $5.0\,\mu g\,ml^{-1}$. 6 validation samples consisted of the analytes without interfering components were designed. The concentrations of analytes in these validation samples were roughly 1.0, 1.5, 2.0, 2.5, 3.5 and $4.0\,\mu g\,ml^{-1}$, respectively. These validation samples were used to check the accuracy of the ATLD model.

2.3.3. Real samples

Two groups of samples were prepared: in the first one, 4 samples were prepared by diluting the dryness of honey samples which was obtained as those employed in Section 2.3.1. Then, these honey samples were diluted to 1 ml with proper amount of working solutions to obtain the concentrations of 100, 200, 350, and $400\,\mu g\,kg^{-1}$. These samples were designed with the purpose of quantifying quinolones in the presence of interfering components in honey matrix. While in the second group, analytes and extracts of honey were mixed and evaporated to dryness, then diluted with 1 ml

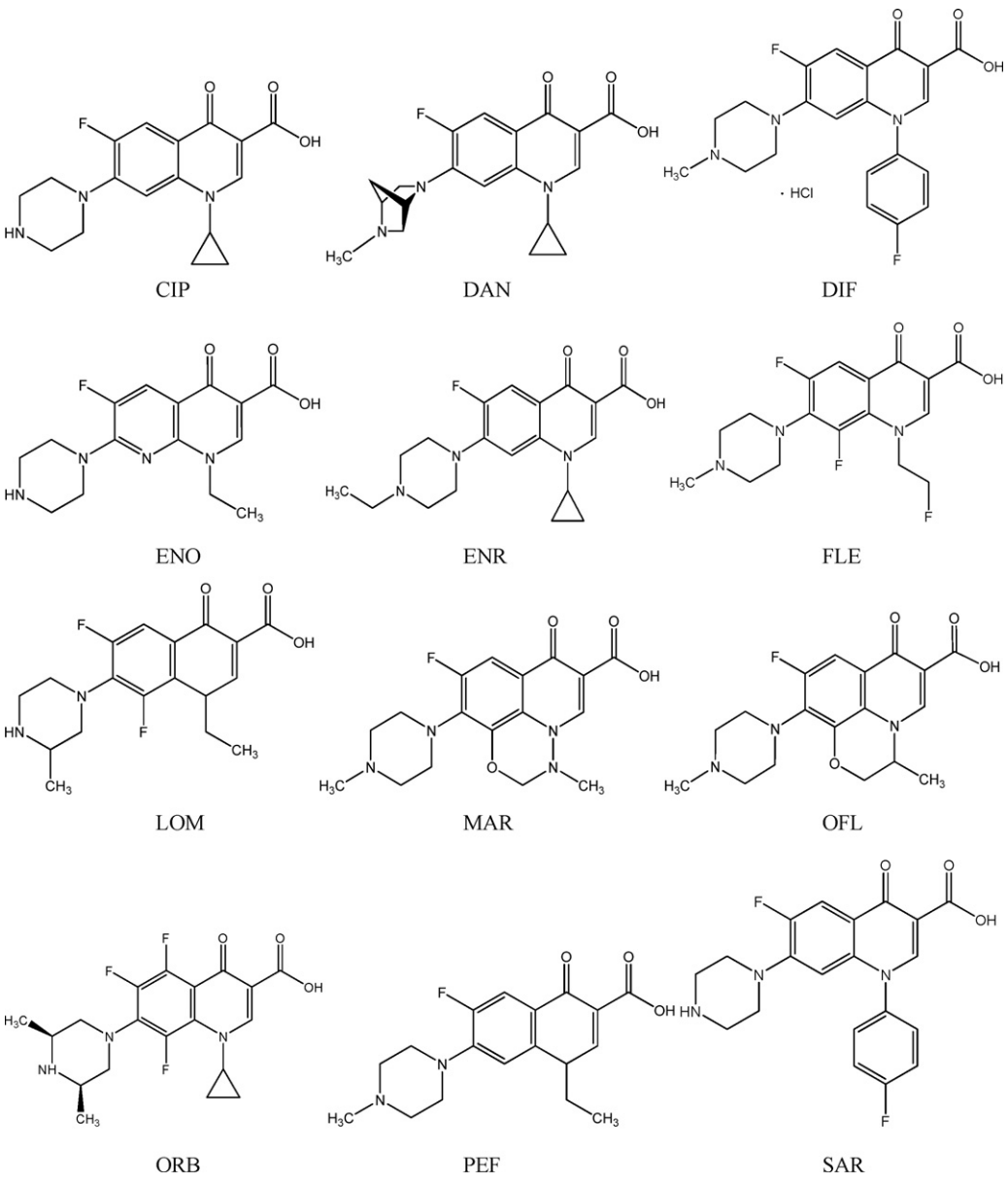

Fig. 1. Structure formulae of the quinolones in this paper.

mobile phase. The main purpose was to check the influences of evaporation procedure. Concentrations in the second group were 150, 250, and 350 $\mu g\,kg^{-1}$.

2.4. Alternating trilinear decomposition (ATLD) method

ATLD [31] method, which was proposed in 1996, has the advantages of being insensitive to component number and converging more effectively. If a sample produces a data matrix with the size of $I \times J$, for example, by HPLC-DAD, then the samples' data set obtained by arranging each of K samples involving calibration and test samples is a three-way array with size of $I \times J \times K$. Such a data array will have a trilinear structure as long as there is no time shift in the data and can be uniquely decomposed by such trilinear decomposition methods such as ATLD, providing the qualitative profiles (**A** and **B**) and the relative concentrations (**C**) of individual components. Then, the linear relationship between the relative concentrations in calibration samples and the real ones of each analyte can be obtained. Finally, the predicted concentrations of the analytes of interest in unknown samples can be obtained by using the corresponding relative concentrations and the former linear relationship. This is the core ideology of second-order advantage, which can quantify analytes of interest even in the presence of uncalibrated interfering components.

2.5. Software

The data were analyzed using Matlab and Three-way Data Analysis Platform Software which was developed by our laboratory based on the presented literatures of these trilinear decomposition algorithms.

3. Results and discussions

Fig. 2 depicts the three-dimensional and contour plots of the complete landscape of UV/visible absorption intensity as a function of time and wavelengths for a mixture of 12 quinolones. As shown in Fig. 2, quinolones elute effectively within 11 min. It is worthy of noting that 5 compounds elute between 4.38 min and 5.98 min although it seems only 4 peaks are visible (see Fig. 2). It

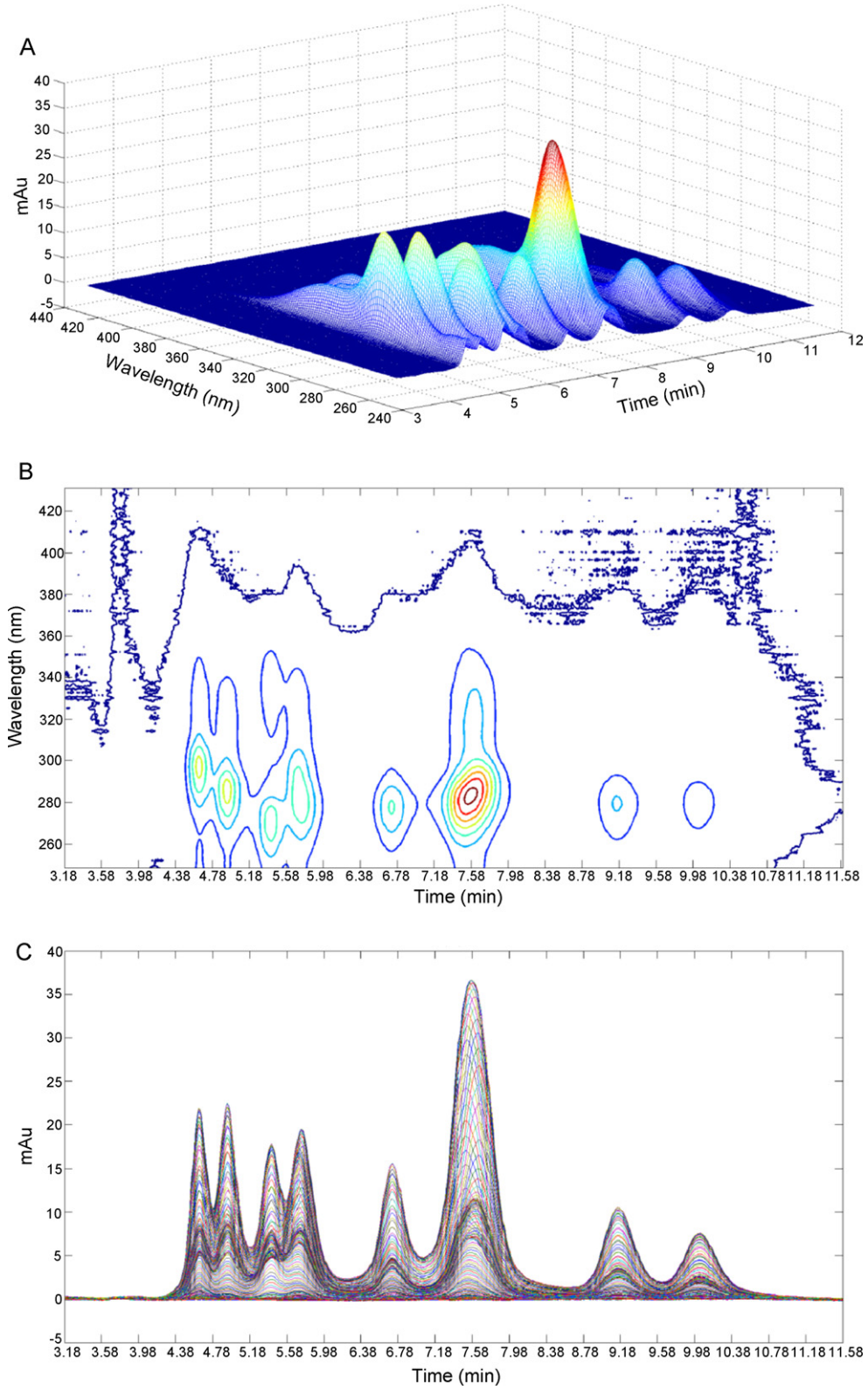

Fig. 2. (A) Three-dimensional plot of a typical chromatography of a sample containing all analytes. (B) The corresponding contour plot. (C) The corresponding two-dimensional plot. All concentrations of analytes are around 3.5 μg ml^{-1}.

is also evident from Fig. 2 that 4 compounds are presented in the region between 7.18 and 7.98 min; however, only a single peak can be obviously observed. Additionally, unexpected components may occur in the real samples and overlap any of the peaks shown in Fig. 2. All the above-mentioned results indicate that it is a much more complex system. Traditionally, univariate methods could not be directly applied to quantify these overlapped analytes. Fortunately, these overlapped peaks can be mathematically separated

Table 1
Elution ranges and the corresponding maximum response positions of analytes.

Analyte	Time ranges (min)	Max position	Analyte	Time ranges (min)	Max position
MAR	4.22–5.51	4.62	ENR	6.51–8.50	7.45
FLE	4.70–5.55	4.95	DAN	6.68–8.55	7.53
ENO	4.74–6.20	5.42	LOM	6.68–8.53	7.65
OFL	4.81–6.65	5.71	ORB	7.39–8.56	7.73
PEF	4.81–6.65	5.80	DIF	8.38–10.43	9.16
CIP	6.40–7.95	6.75	SAR	8.95–11.38	10.06

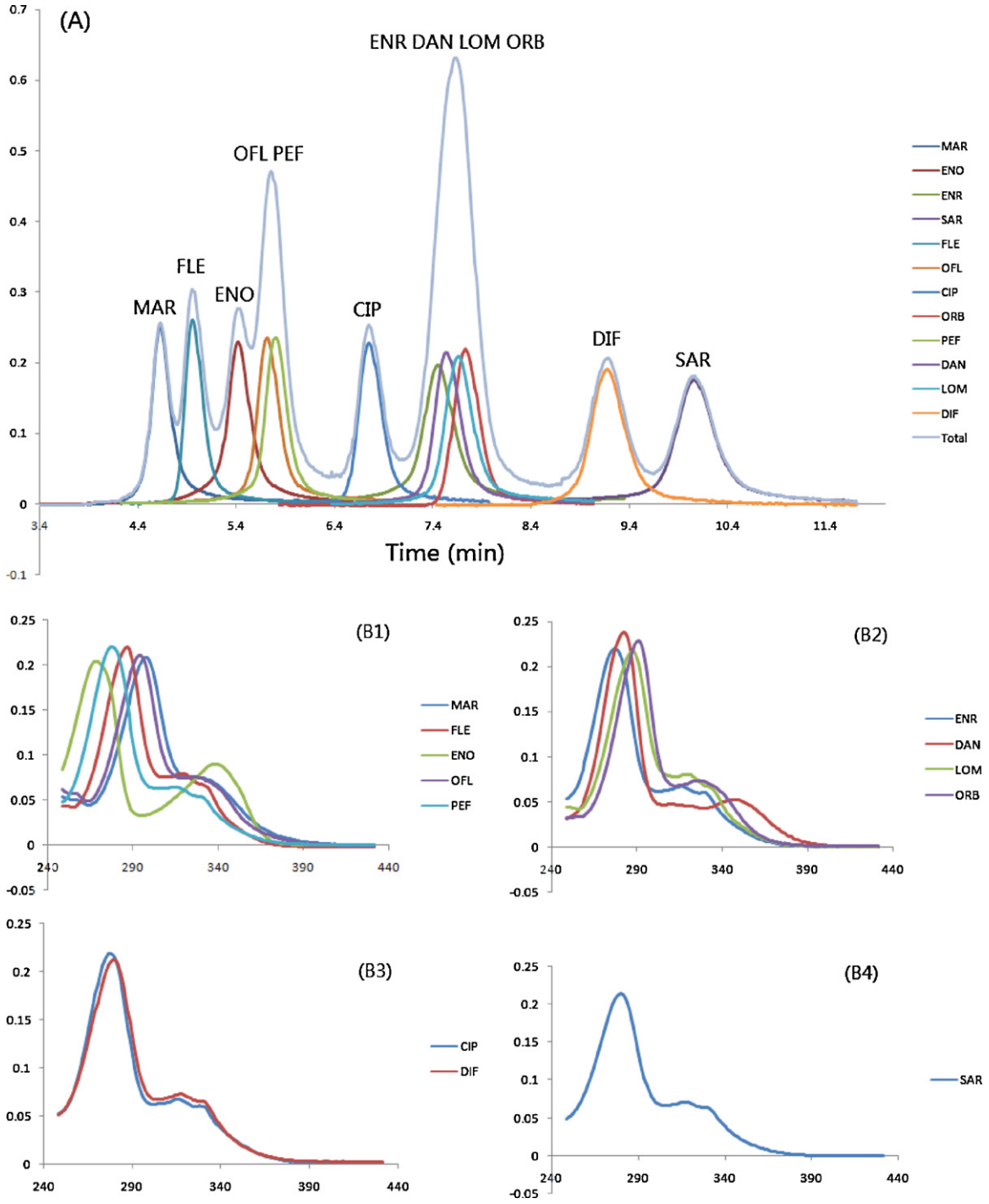

Fig. 3. Resolved chromatographic peaks (A) and the corresponding spectra (B1)–(B4) of 12 quinolones. "Total" represents the mixture peak. All analytes' profiles were normalized.

with the aid of second-order chemometric methods. For example, trilinear methods which have the second-order advantage that can quantify analytes even in the presence of uncalibrated interfering components can be employed to overcome such problems. In this paper, ATLD was employed to decompose the three-way data.

3.1. Validation samples

The validation samples were designed to check the validity of ATLD method. Elution regions of analytes are shown in Table 1. Values of elution time range of analytes shown in Table 1 and the chromatographic profiles depicted in Fig. 2 indicate clearly that MAR, FLE, ENO, OFL and PEF are co-elution compounds and chromatographic profiles of DAN, LOM, ORB and ENR are seriously overlapped. Such a chromatographic system is a great challenge for analysts. The common way of resolving serious overlapped peaks resorts to a long time separation and/or employs more complex chromatographic methods.

However, there is another way of resolving these overlapped peaks that resorts to mathematical separation by using chemometric methods such as ATLD. It is important to note that the traditional way of decomposing the whole data array with a proper number of components is not suitable for simultaneously quantifying all of the analytes. Actually, quinolones were resolved independently according to their elution region in this work. From Fig. 2, it is apparent that the elution profiles of analytes can be briefly classified as four regions based on the elution time: (I) where MAR, FLE, ENO, OFL and PEF were co-eluted; (II) where only a single analyte CIP was presented; (III) where four anlaytes ENR, DAN, LOM and ORB existed; and (IV) where DIF and SAR were successfully separated. From the traditional chromatographic standpoint, only those, CIP DIF and SAR which were isolated can be accurately quantified by univariant methods, leaving the results of the remaining analytes definitely unacceptable. Fortunately, these overlapped peaks can be mathematically separated with the aid of second-order chemometric methods. The mixture profiles of analytes and their corresponding resolved profiles using alternating trilinear decomposition algorithm are depicted in Fig. 3. The quality chromatographic profiles illustrated obviously that overlapped peaks of analytes in the elution I and III could been successfully separated using ATLD.

Table 2 shows the added and resolved concentrations as well as the corresponding statistical index (root mean squared errors of prediction, *RMSEP*) for the analyzed quinolones. *RMSEP* and recovery as well as standard derivation turn out that the quantitative results of each of quinolones obtained from ATLD are acceptable, which further verifies the accuracy of the trilinear decomposition method on the resolution of overlapped peaks of analytes. In addition, the strategy that employs second-order instruments coupled with second-order calibration based on alternating trilinear decomposition principle has shown the potential ability of resolving overlapped chromatographic peaks and has been a subject of current interest.

3.2. Real samples

Honey sample has shown a complex chromatographic background. Usually, there are a lot of interfering components which will absolutely have influences on the quantitation of the analytes. Hence, trivial work is required to eliminate the effects of interfering components. However, with the aid of trilinear decomposition, analytical procedure can be significantly improved in terms of efficiency and simplicity. Fig. 4 depicts the three-dimensional HPLC-DAD profiles of a honey sample (Fig. 4A), the contour plots of the landscape of honey sample (Fig. 4B), three-dimensional plot of a mixture of 12 quinolones and honey background (Fig. 4C) and the corresponding contour plot (Fig. 4D). Obviously, almost all of

Table 2
Recovery study of mixtures of the analytes in validation samples.

	Taken ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	RMSEP[a]	Recovery[b]
MAR	1.00	0.93	0.26	105.7 ± 7.3%
	1.50	1.61		
	2.00	2.24		
	2.50	2.56		
	3.50	3.71		
	4.00	4.53		
ENO	1.00	0.90	0.27	104.4 ± 9.0%
	1.51	1.60		
	2.01	2.25		
	2.51	2.51		
	3.51	3.67		
	4.01	4.58		
PEF	1.00	1.09	0.25	99.2 ± 9.7%
	1.50	1.39		
	2.00	2.09		
	2.50	2.69		
	3.49	2.97		
	3.99	3.80		
ENR	1.00	0.91	0.27	104.8 ± 10.8%
	1.51	1.75		
	2.01	2.19		
	2.51	2.31		
	3.51	3.96		
	4.01	4.33		
LOM	1.00	0.99	0.19	99.5 ± 6.6%
	1.50	1.48		
	2.00	2.09		
	2.50	2.28		
	3.49	3.27		
	3.99	4.35		
DIF	1.00	1.09	0.15	100.6 ± 7.0%
	1.50	1.56		
	1.99	2.11		
	2.49	2.31		
	3.49	3.23		
	3.99	3.93		
FLE	1.00	1.04	0.21	106.8 ± 3.4%
	1.50	1.60		
	2.00	2.24		
	2.50	2.60		
	3.50	3.66		
	4.00	4.39		
OFL	1.00	1.00	0.20	102.3 ± 5.8%
	1.50	1.49		
	2.01	2.18		
	2.51	2.45		
	3.51	3.42		
	4.01	4.45		
CIP	1.00	1.02	0.16	100.0 ± 6.1%
	1.51	1.50		
	2.01	2.16		
	2.51	2.31		
	3.51	3.31		
	4.02	4.22		
DAN	1.00	1.04	0.20	104.1 ± 6.2%
	1.50	1.61		
	2.00	2.19		
	2.50	2.35		
	3.51	3.52		
	4.01	4.41		
ORB	1.00	1.05	0.19	99.8 ± 8.3%
	1.50	1.34		
	1.99	2.12		
	2.49	2.64		
	3.49	3.11		
	3.99	4.12		
SAR	1.00	0.95	0.19	104.5 ± 7.7%
	1.51	1.63		
	2.01	2.33		
	2.51	2.54		
	3.51	3.51		
	4.01	4.30		

[a] Root mean squared error of prediction, $\mathrm{RMSEP} = \sqrt{\sum_{n=1}^{N}(x-\hat{x})^2/N-1}$.

[b] Average recovery and standard derivation.

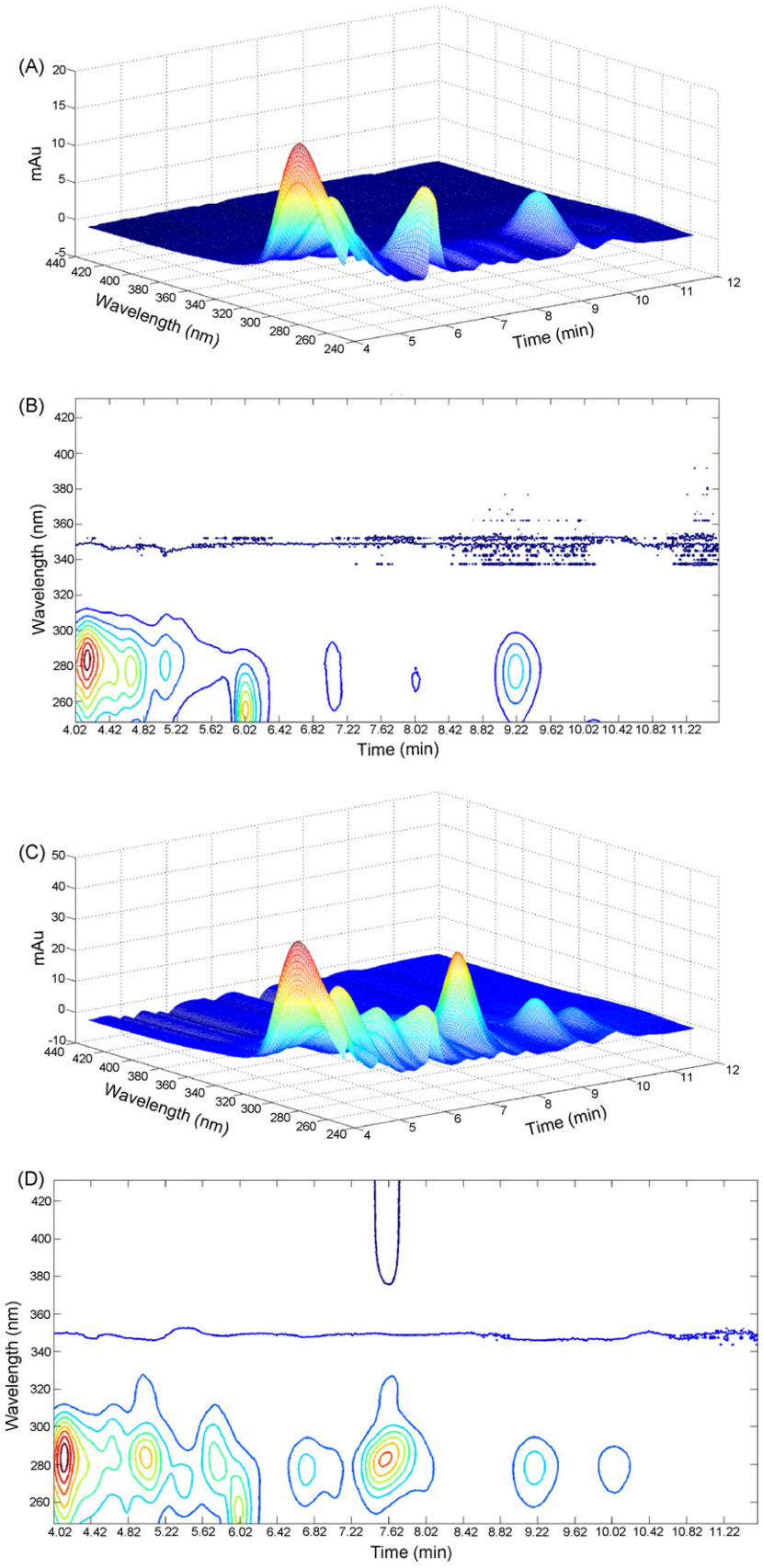

Fig. 4. (A) Three-dimensional plot of a honey sample and (B) the corresponding contour plot. (C) Spiked honey sample with analytes and (D) the corresponding contour plot. The concentrations are the same as those shown in Fig. 2.

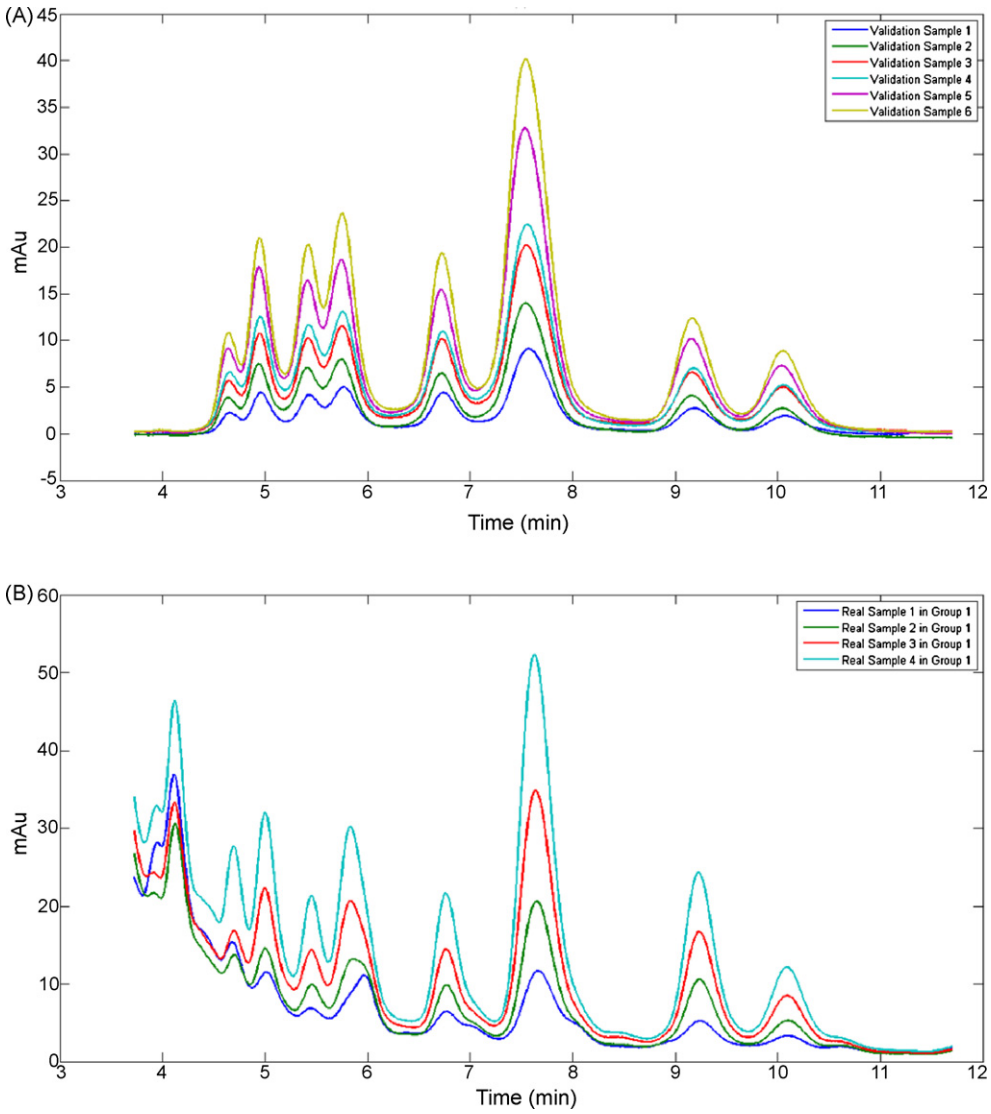

Fig. 5. Chromatographic profiles of mixture of analytes (A) and the mixture of analytes and honey samples (B). Validation sample 1–6 represent the 1–6 levels of concentration of analytes in validation samples, respectively and the corresponding concentrations are roughly 1.0, 1.5 2.0 2.5, 3.5 and 4.0 μg ml^{-1} for all the analytes, respectively. Real sample 1–4 in Group 1 represent 1–4 levels of concentrations of analytes in the first group of real samples, respectively and the corresponding concentrations are roughly 1.0, 2.0, 3.5 and 4.0 μg ml^{-1} for all of analytes (which are corresponding to 100, 200, 350, and 400 μg kg^{-1}, respectively) respectively. All shown data were recorded at 276.4 nm.

the analytes are overlapped by honey background, especially for ENR, DAN, LOM, and ORB which are completely covered by interfering components. In this kind of situation, methods based on local rank constraints and selective information can hardly provide valid results. Additionally, the preestimated number of components is also a crucial parameter for all of the second-order calibration methods. It is a little pity that the current methods for estimating the number of components cannot provide valuable information for such a complex data array. Hence, method which has the advantage of being insensitive to the preestimated number of components is of importance. Furthermore, the decomposition efficiency is also an important consideration for this type of large data set. In fact we also tested the performance of other methods for analyzing the data, such as self-weighted alternating trilinear decomposition (SWATLD) [36], alternating penalty trilinear decomposition (APTLD) [37]. Finally, ATLD could provide a good result of quantifying analytes of interest even in the presence of uncalibrated interfering components.

Whether the analyzed three-way data array has a trilinear structure is of importance. However, it is common to find time shifts in the data, particularly when the analyzed data have a complex background. If there are indeed time shifts in the data, it should be processed before trilinear decomposition method employed [38,39]. Fig. 5 shows the chromatographic peaks in validation samples (A) and real samples (B) to investigate the repeatability of retention time of various analytes. It can be clearly found that there seems to be no obvious time shifts in both types of validation samples and real samples. Moreover, a comparison between Fig. 5A and B indicates that there is a clear intensity increment in each of the concentration levels in Fig. 5B, suggesting that most elution regions of analytes are overlapped by honey background, which agrees well with those shown in Fig. 4. According to our experience, these analytes cannot be accurately quantified by tradition chromatographic methods which using calibration curves quantify concentrations of analytes. Therefore, methods which have the second-order advantage that quantify analytes of interest in the presence of uncalibrated interfering components are preferred. In this paper, we select ATLD as an example of interpreting the HPLC-DAD data.

At first, the obtained data were directly decomposed using ATLD, all of the analytes were successfully resolved except for CIP and DIF. It is interesting to find that the resolved spectral profiles of CIP and

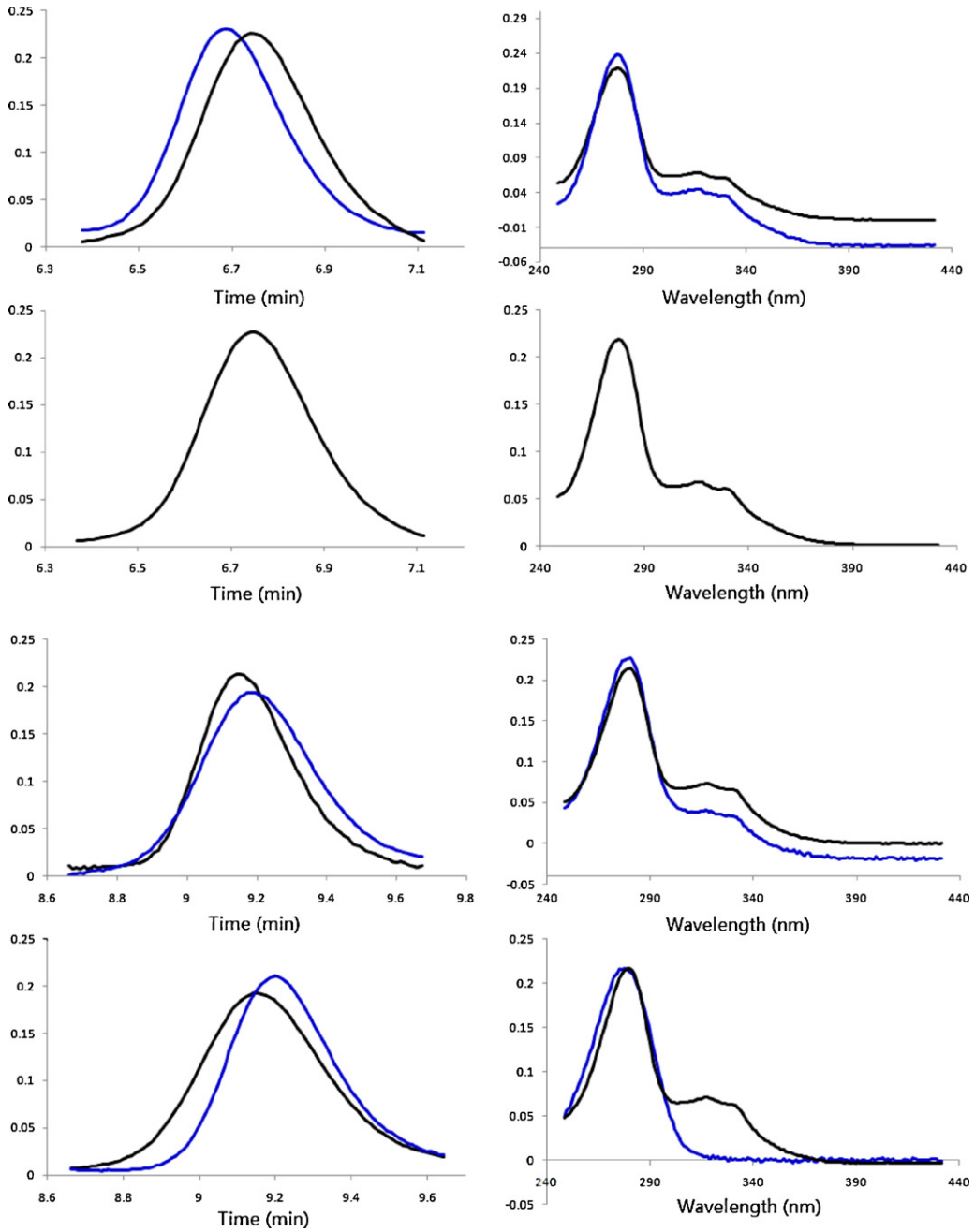

Fig. 6. First row: analyze original data of CIP and second row: after time shifts being corrected. Third row: analyze original data of DIF and fourth row: after time shifts being corrected. Blue lines represent interferents and black lines represent compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

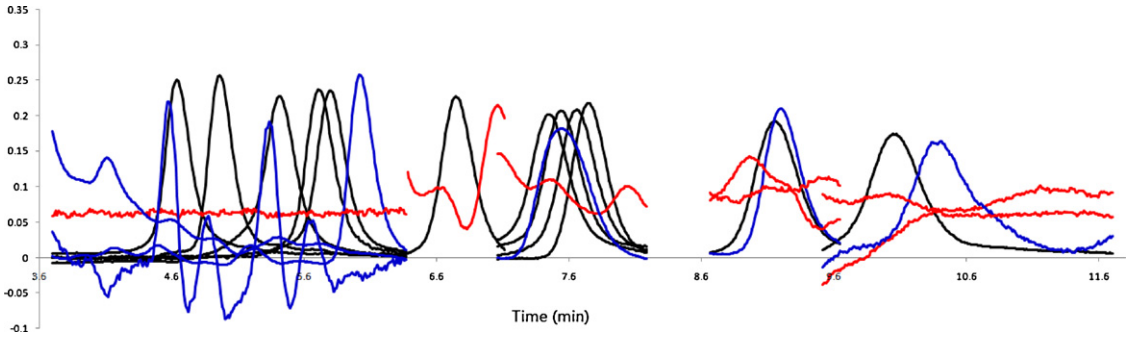

Fig. 7. Resolved profiles of analytes (black lines), interferents (blue lines) and backgrounds (red lines) in real samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Recovery study of mixtures of analytes in the first group of real samples.

	Taken ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	RMSEP[a]	Sig.[b] (2-tailed)
MAR	1.00	0.90	0.18	0.46
	2.00	1.99		
	3.50	3.77		
	4.00	4.11		
ENO	1.01	1.02	0.31	1.00
	2.01	1.94		
	3.52	3.18		
	4.02	4.43		
PEF	1.00	0.99	0.06	0.26
	2.00	2.02		
	3.49	3.43		
	3.99	3.93		
ENR	1.00	0.96	0.41	0.14
	2.01	2.11		
	3.51	3.75		
	4.01	4.33		
LOM	1.00	1.05	0.25	0.74
	2.00	2.10		
	3.49	3.47		
	3.99	3.77		
DIF	1.00	1.04	0.16	0.92
	1.99	1.99		
	3.49	3.36		
	3.99	4.06		
FLE	1.00	1.01	0.13	0.77
	2.00	1.91		
	3.50	3.46		
	4.00	4.20		
OFL	1.00	0.98	0.26	0.60
	2.01	2.08		
	3.51	3.33		
	4.01	4.42		
CIP	1.00	1.01	0.06	1.00
	2.01	1.96		
	3.51	3.49		
	4.02	4.08		
DAN	1.00	1.05	0.29	0.96
	2.00	2.03		
	3.51	3.65		
	4.01	3.77		
ORB	1.00	1.06	0.22	0.78
	1.99	2.01		
	3.49	3.54		
	3.99	3.78		
SAR	1.00	0.98	0.22	0.61
	2.01	2.00		
	3.51	3.46		
	4.01	4.23		

[a] Root mean squared error.
[b] Significance values obtained from SPSS software, the values larger than 0.05 means than there is no significant difference.

Table 4
Recovery study of mixtures of analytes in the second group of real samples.

	Taken ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	RMSEP	Sig. (2-tailed)
MAR	1.50	1.27	0.19	0.42
	2.50	2.34		
	3.50	3.57		
ENO	1.51	1.49	0.15	0.25
	2.51	2.47		
	3.52	3.31		
PEF	1.50	1.57	0.35	0.30
	2.50	2.24		
	3.49	3.08		
ENR	1.51	1.18	0.59	0.66
	2.51	1.89		
	3.51	3.96		
LOM	1.50	1.10	1.41	0.14
	2.49	1.68		
	3.49	1.72		
DIF	1.50	1.71	0.16	0.23
	2.49	2.50		
	3.49	3.58		
FLE	1.50	1.52	0.07	0.65
	2.50	2.45		
	3.50	3.59		
OFL	1.50	1.42	0.31	0.77
	2.51	2.36		
	3.51	3.91		
CIP	1.51	1.64	0.18	0.77
	2.51	2.49		
	3.51	3.30		
DAN	1.50	1.54	0.11	0.89
	2.50	2.40		
	3.51	3.61		
ORB	1.50	1.57	0.26	0.90
	2.49	2.67		
	3.49	3.18		
SAR	1.51	1.38	0.18	0.63
	2.51	2.58		
	3.51	3.72		

DIF are quite similar to their corresponding interferents, respectively, see the first and third rows shown in Fig. 6. As shown in Fig. 6, more than one pseudo components were covered for a single component in case of using trilinear decomposition to analyze the time-shifts data. This conclusion was clearly confirmed by the profiles shown in Fig. 6: two chromatographic and spectral profiles were obtained for both CIP and DIF. An investigation of Fig. 6 shows that CIP seems go left and DIF goes right in the presence of honey background. To make the data have a low-rank trilinear structure, time shifts of CIP and DIF were corrected manually according to calibration samples: the way is roughly based on the maximum position of CIP and DIF. Finally, chromatographic profiles of CIP and DIF were recovered by ATLD (in the second row of Fig. 6 the interfering component, which is shown in Fig. 7, is omitted for the sake of convenient vision). Additionally, an interfering component appeared in the elution range of DIF (see the fourth row of Fig. 7). By the way, there is another way of handing with the data having time shift problems which resorts to the methods which require only bilinear structure, for example, MCR-ALS, HELP, WFA. But we should keep awareness that these methods may face the rotational ambiguity problem.

The resolved profiles of quinolones, interfering components and backgrounds are depicted in Fig. 7. One can find that each of analytes, which was seriously overlapped with the matrix of honey sample, has been successfully recovered from overlapped peaks using ATLD. In the first elution region where MAR, FLE, ENO, OFL and PEF are presented, more than one interfering components were co-eluted in honey sample. Three additional components were used to model the uncalibrated interfering components. In contrast to several interfering components appeared in the first elution region, it seems that only one interfering component eluted in the elution region of ENR, DAN, LOM and ORB. However, all of four analytes are overlapped by the interfering component and, more seriously, it is hard to find selective information for any of four analytes. Fortunately, with the aid of trilinear decomposition, both the chromatographic and spectral profiles were successfully recovered. The elution profiles of analytes are in good agreement with those shown in Fig. 3(A) (the spectra of analytes and interfering components are omitted for the sake of simplicity).

Quantitative information and the corresponding significance statistical parameters for each of analytes in the first group of real samples are presented in Table 3. Statistical parameter for each of analytes is larger than 0.05, indicating that no significance difference between the actual values and the resolved ones. Combining with the resolved profiles shown in Fig. 7, a simple conclusion that even in the presence of unknown components in complex background, both qualitative and quantitative information of the analytes of interest has been extracted by ATLD. This is the well-known “second-order advantage”.

Table 5
Analytical parameters for analytes in honey samples.[a]

	MAR	FLE	ENO	OFL	PEF	CIP
LOD	9.7	1.3	0.01.5	0.4	1.8	1.9
LOQ	29.3	4.1	4.6	1.2	5.5	5.8

	ENR	DAN	LOM	ORB	DIF	SAR
LOD	2.6	2.8	4.9	0.5	18.7	11.6
LOQ	8.0	8.6	14.9	1.5	56.7	35.1

[a] Concentration unit: $\mu g\,kg^{-1}$.

In the second group of real samples, analytes and extracts of honey were mixed before being evaporated. Quantitative information for each of compounds is listed in Table 4. Obviously, the results are acceptable. However, the quantitative results are little worse compared with the values shown in Table 3, especially for LOM. It is possible that LOM is lost in the evaporated procedure. In Table 5, limit of detection (LOD) and limit of quantitation (LOQ) are shown (LOD and LOQ were calculated according to the ref. [40]). The values of LOQ were from 1.2 $\mu g\,kg^{-1}$ for OFL to 56.7 $\mu g\,kg^{-1}$ for DIF. These low concentrations are below MRLs established by the China. The above-mentioned results indicated that trilinear decomposition is a useful tool for interpreting overlapped peaks in chromatographic data. Second-order instrument coupled with trilinear decomposition can be used to separate overlapped peaks and fully extending the second-order advantage that quantify analytes in the presence of uncalibrated interfering components. In addition, the strategy employed in this paper can be considered as an example of application of trilinear decomposition methods in analyzing analytes in complex samples; more practical work is of course needed for analysts in the future.

4. Conclusions

A new strategy that combines second-order calibration methods based on alternating trilinear decomposition with high performance liquid chromatography instruments with DAD has been applied to quantify 12 quinolones in honey samples. Overlapped peaks have been successfully resolved and the quantitative results of the analytes of interest have been accurately estimated. This novel method has the advantage of more effective and simpler chromatographic condition compared with traditional chromatographic methods. Additionally, the strategy developed in this paper can be treated as an example of interpreting three-way data from second-order analytical instruments. Moreover, another attractive aspect in this work is that the developed strategy can be directly applied to other complex systems, for example, analyzing multi-residues in environmental samples.

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