Regular Article

ANALYTICAL
AND BIOANALYTICAL
CHEMISTRY
RESEARCH

Published by the
Iranian Chemical Society

***Anal. Bioanal. Chem. Res.**, Vol. 2, No. 2, 129-137, December 2015.*

Coupling Second-Order Excitation-Emission Spectrofluorimetric Data with Standard Addition method to Quantify Carvedilol in Real Samples

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(Received 4 September 2015, Accepted 23 November 2015)

Prediction using pure standards is expected to be biased whenever the slope of the calibration is affected by the presence of sample matrix. Moreover, in the presence of unknown spectral interferents, first-order algorithms like partial least squares cannot be used. In this study, a method for determination of carvedilol (CAR) in tablet and urine samples is proposed by excitation-emission fluorescence spectroscopy (EEM). The multivariate curve resolution-alternating least-squares (MCR-ALS) coupled with trilinearity constraint exploiting the second order advantage is applied for the analysis of EEMs. Moreover, the combination of standard addition with MCR-ALS was used to correct the matrix effect. Indeed, by the proposed strategy, both matrix effect and the problem of the presence of unknown interferents in determination of CAR are overcome.

The MCR-ALS method was applied on EEMs under non-negativity and trilinearity constraints. For both samples, CAR was quantified at low mg l^{-1} level with an overall prediction error of -3.1% and -4.0% in urine and tablet samples, respectively.

Keywords: Carvedilol, Excitation-emission fluorescence, Multivariate curve resolution, Urine samples

INTRODUCTION

Carvedilol, 1-(4-carbazolyloxy)-3-[2-(2-methoxy) ethyl-amino]-2-propanol (Fig. 1), is a non-selective β-adrenergic receptor antagonist and a α1-adrenoceptor blocker. The β1-blockade produces a decrease in heart rate and in the force of contraction of the cardiac muscle [1-3].

The fluorescence properties of CAR have been exploited for analytical purposes [4]. Spectrofluorometric method has a wide application in analytical chemistry because of its inherent sensitivity [5]. The use of spectrofluorometric method for determining drugs in biological fluids is difficult due to the presence of natural fluorescent interferents which reduce the selectivity of the method because of extensive spectral overlap or the presence of matrix interferents. The situation can be more complicated when the spectral interferents are unknown. This frequently occurs in spectrofluorometric determinations in biological samples.

In the last years, different strategies have been proposed to circumvent this problem by combining spectro-fluorometric data and three-way chemometric tools such as parallel factor analysis (PARAFAC) [6-10], alternating trilinear decomposition (ATLD) [11], self-weighted alternating trilinear decomposition (SWATLD) [12], alternating penalty trilinear decomposition (APTLD) [13] and multivariate curve resolution-alternating least squares (MCR-ALS) coupled with trilinearity [14-22]. The MCR-ALS method makes the quantification possible even if the unknown interferents are present in the prediction samples. The property was called second-order advantage [23]. MCR-ALS has been also applied to investigate the multi equilibrium systems using spectroscopic titrations (fluorescence, UV-Vis absorption, *etc*.) [24], the resolution of multiple coeluted peaks in chromatography [25] and multidimensional spectroscopy [26-28].

The objective of this work is the development of a method for direct determination of CAR in pharmaceutical tablets and human urine samples using standard addition and excitation-emission (EEM) fluorescence data. This combination has been called SOSAM [29,30]. The three-

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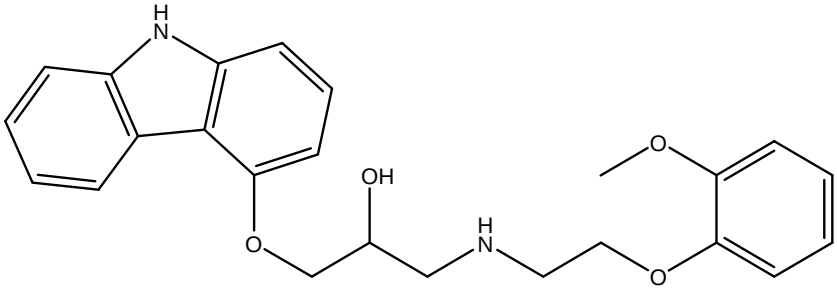

Fig. 1. Chemical structure of carvedilol.

way data are arranged as two-way data and analyzed by MCR-ALS exploiting second-order advantages.

EXPERIMENTAL

Apparatus and Software

Spectrofluorimetric EEM spectra were recorded by a JASCO spectrofluorimeter (FP 6200) equipped with a xenon discharge lamp with a 1 cm quartz cell. A JENWAY ion-meter, Model 3345 was used for pH adjustment.

The treatment of the EEM data was performed in MATLAB 6.5 environment (MATLAB 6.5, The Mathworks Inc., Natick). MCR-ALS method is implemented in a small set of MATLAB functions (http://www.ub.es/gesq/mcr/mcr.htm).

Reagents and Solutions

Carvedilol (chemical correspondent product) was used as standard (99.98%). Other reagents were of analytical reagent grade. Doubly distilled water was used in all experiments. Stock standard solution of carvedilol (100.0 mg l^{-1}) in methanol was used in preparing working solutions. Buffer solution with pH = 3.0 was prepared by sodium acetate/acetic acid system.

Pharmaceutical Preparation (Tablets)

Ten tablets were finely grounded and mixed. An accurately weighed quantity of the mixed powder equivalent to one tenth of the weight of a tablet was transferred into 10.0 ml volumetric flask and made up to the mark with methanol. The content was shaken for 30 min. The resulting solution was filtered and transferred quantitatively into another 10.0 ml volumetric flask and completed to the mark with methanol. For the measurement, 24 µl of this solution was transferred into 10.0 ml volumetric flasks and appropriate increasing amounts of carvedilol standard solution were added based on Table 1. After adding 2.0 ml of buffer solution with pH = 3.0, the mixtures were diluted to the mark with doubly distilled water and shacked well.

Procedure for Spiked Human Urine Samples

5.0 ml aliquots of human urine samples were spiked with 0.1 mg l^{-1} of carvedilol. A volume equivalent to 1.0 ml of the resulting urine solution was transferred into 10.0 ml volumetric flasks and 2.0 ml of buffer solution with pH = 3.0 was added to them. To this mixture, standard addition was performed by adding appropriate amounts of the carvedilol stock solution based on Table 1.

Data Sets under Study

For each sample, EEM spectra were recorded at excitation wavelengths (λ_{ex}) range 230-358 nm at regular steps of 4 nm and the emission wavelengths (λ_{em}) range 230-500 nm at 1 nm intervals. Typical EEM fluorescence data for tablet and urine samples have been shown in Figs. 2 a and b, respectively.

However, limited ranges of emission wavelengths were selected and used during MCR-ALS analysis.

THEORY

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

MCR-ALS was used to resolve the pure analyte

Table 1. Standards of Carvedilol Added to the Pharmaceutical and Urine Samples in the Standard Addition Method

Tablet sample	CAR ($mg\ l^{-1}$)	Urine sample	CAR ($mg\ l^{-1}$)
T0	0.00	**U0**	0.00
T1	0.10	**U1**	0.05
T2	0.15	**U2**	0.10
T3	0.20	**U3**	0.15

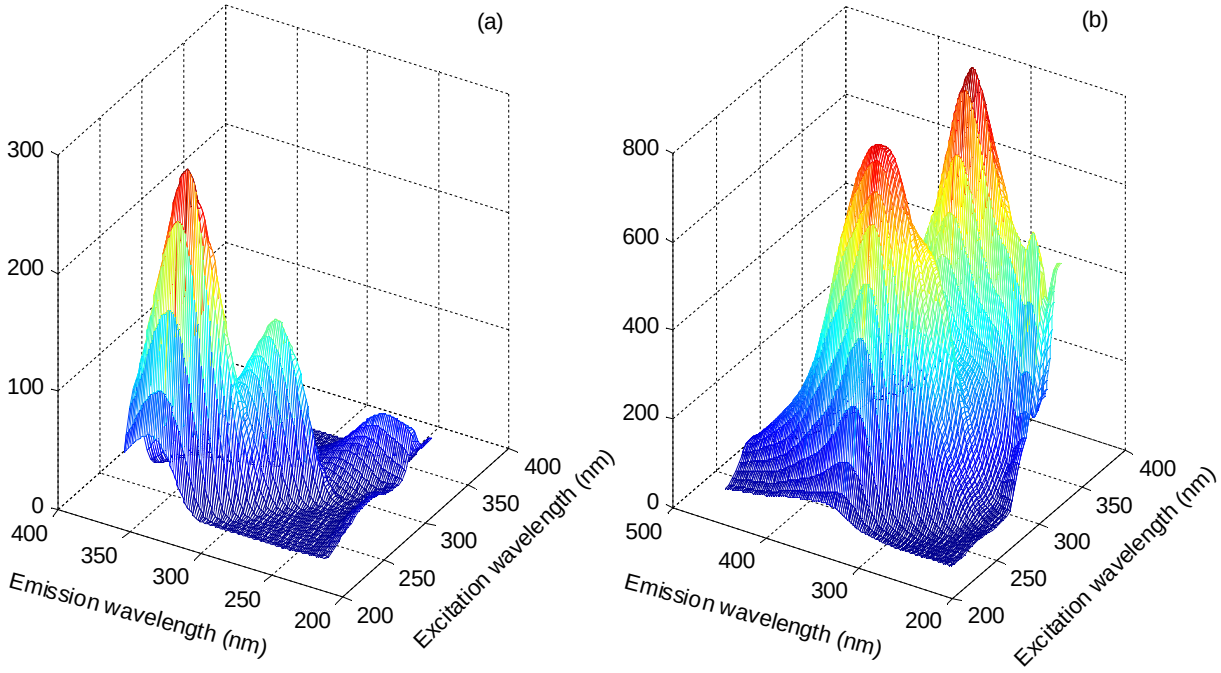

Fig. 2. EEM data for (a) tablet after addition of 0.15 $mg\ l^{-1}$ and (b) urine after addition of 0.20 $mg\ l^{-1}$ of CAR.

responses (*i.e.* excitation spectrum and emission spectrum) from the analysis of the EEM data matrices. MCR-ALS is based on a linear model which assumes additivity of the response of all fluorescent components of the sample. In this resolution method, both excitation-wise and emission-wise matrix augmentations are possible. In the excitation-wise augmentation, matrices are arranged by keeping the common excitation wavelengths in the same row. In the emission-wise (column-wise) augmentation, each emission wavelength is kept in common.

The excitation-wise (column-wise) augmented data matrices (Y_{aug}) are modeled with the equation

$$\mathbf{Y}_{aug} = (\mathbf{S}_{em} \odot \mathbf{C}) \cdot \mathbf{S}_{ex}{}^{T} + \mathbf{E} \quad (1)$$

where S_{ex} and $S_{em,aug}$ are the matrices of excitation and emission spectra of the detected components, respectively, with dimensions $c \times nex$ and $nem \times c$ and **E** is the residual matrix. *c*, *nex* and *nem* are the number of components, number of excitation wavelengths and number of emission wavelengths, respectively. Correspondingly, Y_{aug} and **E** have the same dimensions as $nem \times nex$.

The aim of MCR-ALS method is to estimate the matrices S_{ex} and S_{em}^{T} from resolving Y_{aug} using an iterative optimization. The ALS optimization was started using initial estimates of the emission spectra of the components present in the experimental response matrices. Different methods can be used for this purpose like simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) which identifies the purest variables [31-33]. In this work, initial estimates of the emission profiles were obtained by SIMPLISMA based on the selection of the purest column or rows in the analyzed matrix. In MCR-ALS algorithm, these estimates were initially used to calculate the excitation spectra as:

$$S_{ex}^{T} = (S_{em}^{T} S_{em})^{-1} S_{em}^{T} Y_{aug} \quad (2)$$

From the calculated emission spectra, new emission spectra can be calculated using the equation:

$$S_{em} = Y_{aug} S_{ex} (S_{ex}^{T} S_{ex})^{-1} \quad (3)$$

These two steps are repeated until an optimal solution is obtained that fulfills the constraints postulated and the established convergence criteria. During the iterative calculations, a series of constraints with the purpose of giving solutions with physical meaning and limiting possible solutions are applied [34,35]. In this work, the applied constraints were (a) non-negativity for both emission and excitation spectra, because both emission excitation spectra must be always positive and (b) trilinearity because of the intrinsic trilinear property of EEMs.

The convergence criterion is the minimum of the residual matrix (**E**). Residual matrix is calculated using Eq. (4):

$$E = Y_{aug} - S_{em} S_{ex}^{T} \quad (4)$$

In order to evaluate the results of MCR-ALS, a parameter called percent lack of fit (LOF%) [36,37] can be calculated using Eq. (5) which explains the difference between the original data set, Y_{aug}, and the reconstructed data obtained by $S_{ex}\ S_{em}^{T}$ product,

$$LOF\% = \sqrt{\frac{\sum_{i,j} e_{ij}^{2}}{\sum_{i,j} x_{ij}^{2}}} \times 100 \quad (5)$$

where e_{ij} and x_{ij} are the elements of the matrices **E** and Y_{aug}, respectively.

RESULTS AND DISCUSSION

To provide a chemically reliable resolution of the real samples, the number of chemical components was estimated by principal component analysis (PCA) [38-40]. Figure 3 illustrates the results applying PCA on the augmented EEM data for tablet and urine samples. Analysis of the EVs apparently shows that the number of components present in the EEM data of urine sample is three and in tablet data is two, which are attributed to CAR and interferents present in the tablet and urine samples.

In the analyzed samples, the second-order data have been collected with standard addition of the analyte. Therefore, in a two-component system (tablet sample: analyte plus an unknown interferent), the resolution is unique [41]. As illustrated in refs. [41,42], the unique

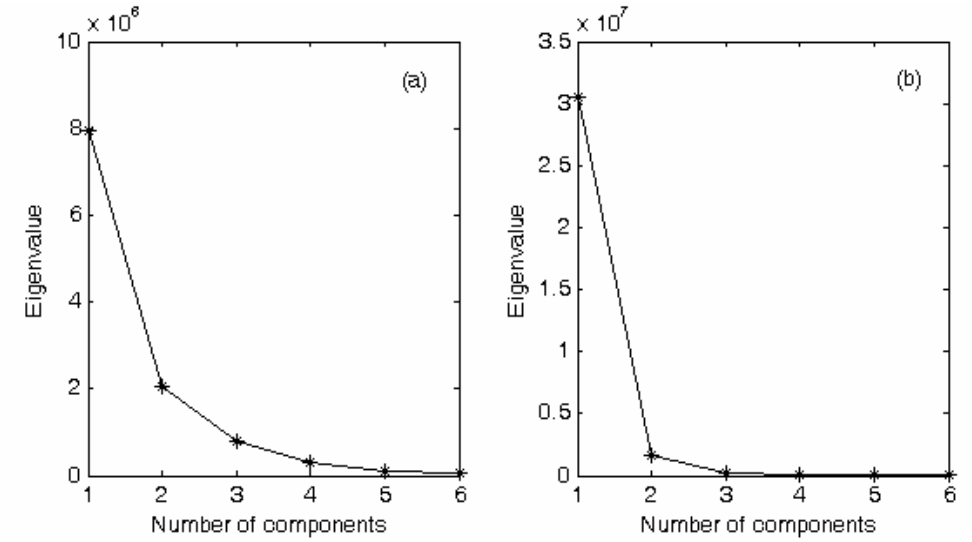

Fig. 3. Variation of EVs with the number of components for EEM data recorded for (a) urine and (b) tablet.

resolution in a three-component system (urine sample: analyte plus two unknown interferents) can be achieved when the selective windows are present for other two modes of all components. In the EEM data of urine sample obtained by standard addition of CAR, the Kruskal condition is not met, because in three-component system, only with adding the standards of analyte, the Kruskal rank of matrix **C** is 1, and therefore sum of the Kruskal rank of matrices **C**, S_{ex} and S_{em}^{T} is lower than $2F+2$. For unique resolution, it should be equal or higher than $2F+2$ [43]. F is the array (Y_{aug}) rank.

Although in the urine EEM data, the profiles may be resolved ambiguously, the quantitative use of the area under the emission profiles in different standard addition EEM data can be made. With increasing concentration, corresponding profiles will grow proportionally.

Analysis of Tablet Samples

The EEMs of the real sample and the spiked samples were augmented in the emission mode and then MCR-ALS under non-negativity and trilinearity constraints was applied on the data. The emission wavelength range 358-450 nm was selected in this analysis. The results of MCR-ALS have been shown in Fig. 4. As can be seen from both excitation and emission spectra in Fig. 4, the interferents are very low in tablet sample analysis. Therefore, it can be concluded that excipients in the analyzed tablet have a little fluorescence signal which cannot interfere in CAR determination, seriously.

For determination of CAR in tablet and urine samples based on the results of MCR-ALS, the relative response for the analyte in different augmented data matrices were plotted versus the added concentration of the analyte. The relative response of the analyte for each sample was calculated from the area under each resolved emission spectrum. Figure 5 depicts standard addition plots for quantification of CAR in tablet. The intercept of the calibration line with the abscissa gives the concentration of analyte in the sample. The correlation coefficient of standard addition plot was 0.9979. The calculated CAR in this sample was 0.288 mg l^{-1} and parameter LOF% of MCR-ALS was 2.7%.

Analysis of Urine Samples

In the analysis of urine sample, MCR-ALS under non-negativity and trilinearity constraints was applied on the augmented data of the EEMs of the real sample and the spiked samples. The selected emission wavelength range

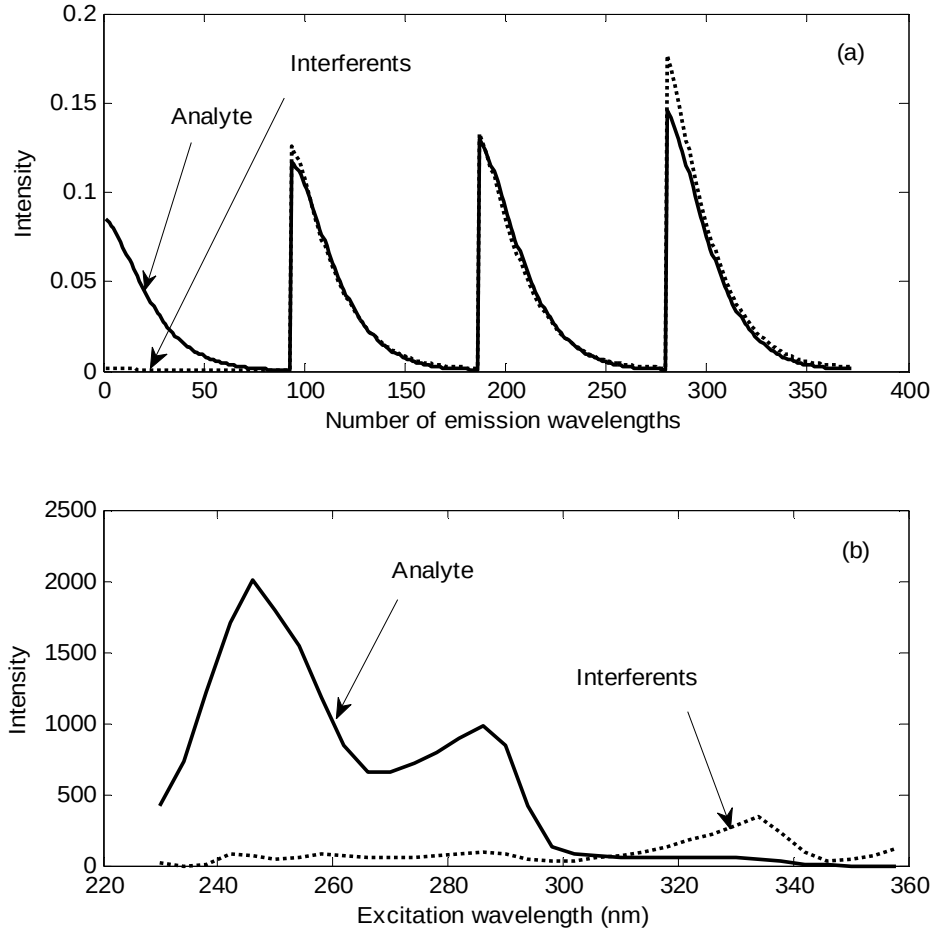

Fig. 4. (a) Emission and (b) excitation spectra resolved by MCR-ALS for analyte and interferent in tablet samples included in Table 1.

was 358-550 nm. The results of MCR-ALS has been illustrated in Fig. 6. As can be seen from the resolved spectra in Fig. 6, the contribution of the unknown interferent in the excitation and emission spectra is very high. Therefore, in urine samples, the effect of interferent is very large. So, CAR determination in urine samples is impossible without using and analyzing the second-order EEM data. Figure 5 shows the standard addition plots for quantification of CAR in urine sample. The correlation coefficient of standard addition plot was 0.9553. The calculated CAR in this sample was 0.097 mg l^{-1} and parameter LOF% of MCR-ALS was 6.8%.

CONCLUSIONS

CAR determination in tablet and urine samples using fluorescence data was shown to involve both matrix effect and unknown spectral interferents. In these conditions, standard addition and second-order data are required to solve the problem. Therefore, excitation-emission fluorescence data of the standard addition samples were collected and analyzed by MCR-ALS.

Quantitation results by MCR-ALS were associated with very low prediction errors. Moreover, the method has no need of long and tedious sample preparation steps.

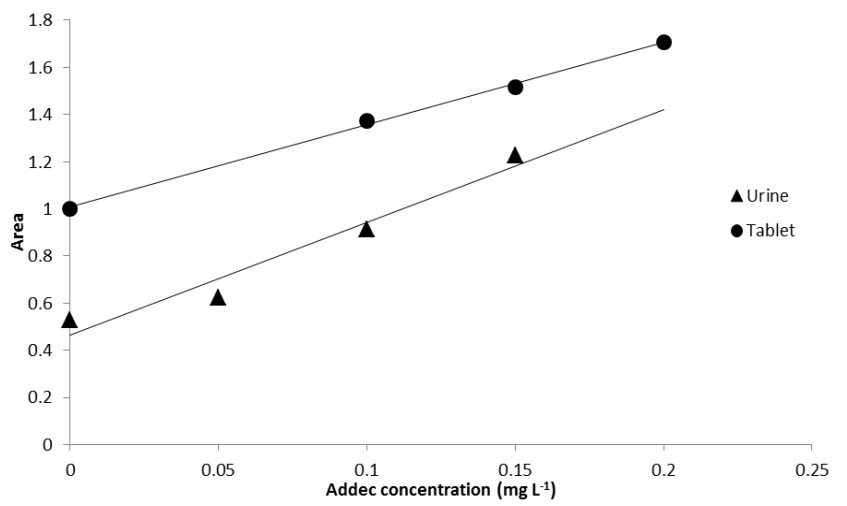

Fig. 5. Standard addition in CAR determination of (●) tablet and (▲) urine samples.

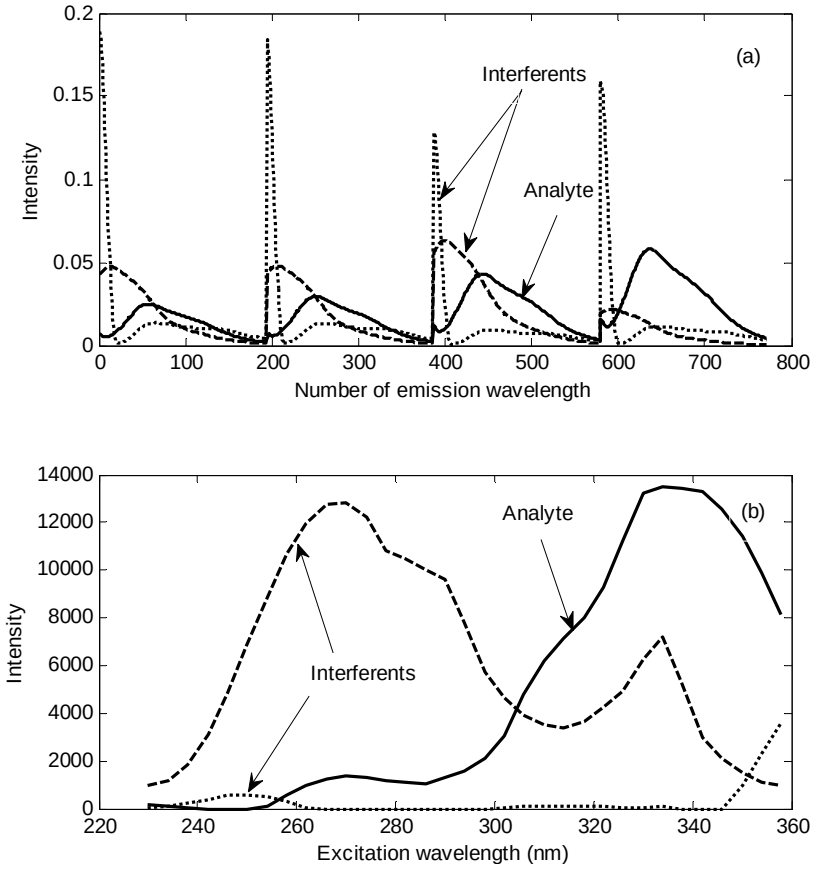

Fig. 6. (a) Emission and (b) excitation spectra resolved by MCR-ALS for analyte and interferents in urine samples included in Table 1.

Therefore, the method is recommended for sensitive determination of CAR in complex samples like urine as an alternative to the expensive methods like HPLC.

ACKNOWLEDGMENTS

The authors acknowledge the Razi University Research Council for support of this work.

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