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Multivariate Curve Resolution-alternative Least Squares for Simultaneous Kinetic–Spectrophotometric Determination of Furosemide and Rizatriptan in Real Samples Based on their Degradation Study

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So far, much research has been devoted to measure drug analytes both in pure form or or in complex matrices, and different methodologies have been employed with their respective advantages and disadvantages. In this work, we introduce an efficient technique, with lower cost and shorter time, using MCR-ALS, for simultaneous determination of Furosemide and Rizatriptan benzoate in their mixtures by analyzing kinetic-spectrophotometric data matrices obtained from monitoring the drug degradation reactions induced by chlorine at the optimized pH. Because of the presence of high spectral overlapping between the analyzed drugs and similar kinetic profiles, the obtained matrix is rank-deficient and hence matrix augmentation was performed for simultaneous analysis of drugs in their mixture. Calibration curves were linear in the ranges 3-50 μM and 2-50 μM for Furosemide and Rizatriptan, respectively. Satisfactory recoveries were obtained and the method showed a good analytical performance toward determination of Furosemide and Rizatriptan in real samples.

Keywords: Furosemide, Rizatriptan, MCR-ALS, Simultaneous, Kinetic-spectrophotometric, Degradation

INTRODUCTION

Determination of drug levels in biological samples is very important from the medical and pharmacological point of view in order to determine the active metabolites, pharmacokinetics, action mechanism, side effects, and toxicity of the prescribed drugs [1,2]. On the other hand, pharmaceutical compounds may be present in surface waters and wastewaters in trace amounts due to inappropriate wastewater treatment processes turning them into the new types of water contaminants with unknown environmental effects and toxicity [3]. It is noteworthy that some of these contaminants may not be completely removed or transformed into other species during the treatment process [4]. Hence, there is a permanent need to accurate, precise, sensitive and selective methods for monitoring these compounds in different biological or environmental samples.

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Furosemide-(5-(aminosulfonyl)-4-chloro-2-[(2-furanyl-methyl)amino]benzoic acid) (Fig. 1a) is a common diuretic drug which is widely prescribed to treat congestive heart failure [5], chronic renal failure [6] and liver cirrhosis [7] as well as a doping agent in athletes for increasing the urine amount as a weight-losing drug or for masking other prohibited drugs [8].

Rizatriptan benzoate (N,N-Dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine benzoate) (Fig. 1b) is a member of the medicine family known as serotonin (or 5HT) agonists commonly prescribed for migraine treatment. Rizatriptan attaches to serotonin receptors in brain which results in narrowing blood vessels and alleviates the pain by decreasing the width of brain blood vessels [9].

A wide variety of analytical methodologies have been used for determination of Furosemide or Rizatriptan in real samples including chromatographic techniques like HPLC and CE [10-15], spectroscopic [16-20] and electrochemical methods [21-25], but to the best of our knowledge there is

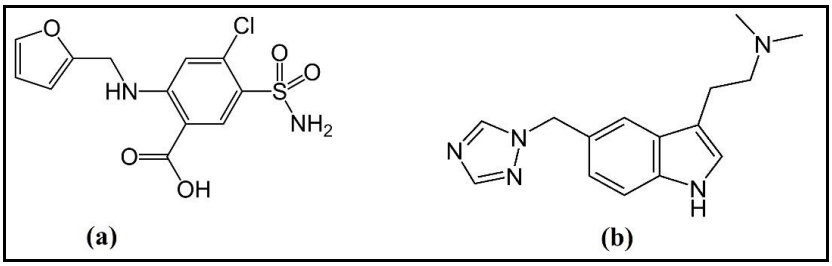

Fig. 1. Chemical structure of Furosemide and Rizatriptan.

no scientific report on their simultaneous determination in the literature.

Kinetic-spectrophotometric methods are useful for multi-component analytical determination in mixtures with high sensitivity and good selectivity. These techniques are based on the reaction of the analytes with a common reagent in a complexation or degradation reaction which is monitored spectrophotometrically.The differences in the reaction rates are employed for the simultaneous determination of the analytes. Traditional spectrophotometric techniques using few wavelengths are inappropriate for multi-component determinations especially when there are multiple components with overlapping spectra. Because these methods provide insufficient information needed for resolving the system. Kinetic-spectrophotometric methods facilitate multi-component determinations by gathering high amounts of information using multiple wavelengths which provide the opportunity of using multivariate chemometric techniques. These techniques have the advantage of exploiting all the information simultaneously and perform accurate analyses in the presence of unknown interferences known as second-order advantage. Among the multivariate methods, MCR-ALS has been previously used for interpretation of drug degradation and complexation kinetics data obtained from spectrophotometric measurements [26-30].

In this study, simultaneous kinetic-spectrophotometric degradation of Furosemide and Rizatriptan benzoate has been studied for drug determination in serum samples using hypochlorite ion as an oxidizing agent at optimum pH conditions and MCR-ALS for resolving the degradation profiles. The slight difference between the rate constants of the reaction of drugs with hypochlorite ions provides the opportunity of using matrix-augmenting MCR-ALS with proper constraints for resolution of kinetic profiles and determination of drugs in the presence of interfering compounds.

THEORETICAL BACKGROUND

When a drug degradation process is monitored by kinetic-spectrophotometric methods, a series of spectra are collected during the time of the reaction which contain the information about the evolution of the components spectra involving in the reaction and contributing to the analytical signal. Accurate separation of the signals of the components depends on the degree of spectral overlapping, similarities between the kinetic profiles and the number of degradation products. Presence of intermediate byproducts with merged concentration profiles increases the difficulty of mathematical signal resolution. Various chemometric methods such as MCR-ALS have been proposed for analyzing such complex systems [27].

MCR-ALS is a commonly used chemometric method to resolve multi-component responses in unresolved mixtures [31]. MCR-ALS is a multivariate curve resolution (MCR) technique using an iterative algorithm to transform a two-way data matrix X to real and chemically meaningful bilinear models where each component has a pure contribution along spectral and concentration directions. This transformation can be written as Eq. (1):

$$X = CS^T + E \quad (1)$$

where C and S^T are the matrices of species concentrations and their corresponding spectra, respectively. E is the part of data inexpressible by the model with experimental error or noise. In ALS algorithm, initial estimates of C or S^T are

taken, and iteratively optimized by the least squares solution and applying proper constraints such as non-negativity, unimodality, equality, closure or local-rank in the optimization process so that the best solution can be obtained and the convergence is achieved. The solutions obtained by MCR methods are often not unique because of the rotational and intensity ambiguities which means that instead of one exact solution there are a set of solutions that fit the experimental data equally well. Using proper constraint along with prior knowledge about the system under study can significantly reduce the rotational ambiguity. The main advantage of MCR-ALS is that it can analyze problematic or rank-deficient data through augmenting a series of data matrices obtained from independent experiments at different experimental conditions resulting in a multiset data. In the case of rank-deficiency, the number of the detected meaningful components in system is lower than the real components which lead to incorrect results of analysis or error in model construction. Rank-deficiency may originate from spectral overlapping of different species in a mixture. The data achieved from standard addition experiment, which is among the most commonly used analytical methods in real sample analysis, is another example of rank-deficiency [32].

It is well-known that data augmentation along the direction of rank-deficiency can eliminate rank-deficiency [31,33-38]. In the case of spectral overlapping, several experiments are performed with different initial concentrations of drugs and the obtained kinetic-spectrophotometric matrices are augmented in the direction of wavelengths. In this case, the single data matrices will have a common direction in which they share their information.

EXPERIMENTAL

Reagents and Solutions

All chemicals were of analytical grade and used without further purification. *Ortho*-phosphoric acid, ammonia, methanol, acetonitrile and sodium hypochlorite (12% v/v) were purchased from Merck (Darmstadt, Germany). Rizatriptan benzoate and Furosemide were purchased form Alborz bulk pharmaceutical company (Saveh, Iran). Blood serum samples were obtained from Imam Khomeini hospital, Urmia, Iran. 0.01 M Phosphate buffer solution with pH 4.0, 0.01 M ammonia buffer with pH 9.6 and 1.5% sodium hypochlorite solution were prepared from their stock solutions. 0.01 M stock solutions of Rizatriptan benzoate and Furosemide were prepared by dissolving proper amounts of drugs in doubly distilled water (DDW) and a 1:1 mixture of methanol and DDW, respectively. All standard and working solutions were then prepared freshly by diluting the corresponding stock solutions with DDW.

Apparatus and Software

All absorption spectra were recorded by Agilent 8453 spectrophotometer equipped with a diode array detector in a 1-cm pathlength quartz cell. A Metrohm 750 pH meter was used throughout the experiments for pH adjustments. Elma Elmasonic E60h ultrasonic bath was used for preparing stock drug solutions. All MCR-ALS calculations were performed in MATLAB 8.1 (MathWorks, Natick, MA, USA) using a freely available graphical package on the web [www.cid.csic.es/homes/rtaqam/tmp/WEB_MCR/download/soft/mcr_toolbox2.zip]. Statistical analysis of data was performed using MS Excel 2013.

Real Sample Preparation

Blood serum samples were collected from healthy volunteers which had not taken drugs before. A 1 ml of serum sample was mixed with 3 ml of acetonitrile for protein precipitation and after centrifugation at 4000 rpm for 10 min, the supernatant was separated and diluted 20 times with DDW. For the preparation of real samples, a 200 µl aliquot of this solution was transferred to a 10-ml volumetric flask and analyzed after spiking with known concentrations of drugs and setting the optimum conditions.

Working Procedure

A series of 50 µM solutions of pure Furosemide and Rizatriptan with different pH values in the ranges 3-9 were prepared for pH optimizations. 3 ml of each solution was transferred to the quartz cuvette and after addition of 100 µl of 1.5% hypochlorite solution, UV kinetic spectra were immediately acquired every 1 min in the ranges 200-400 nm with 1 nm interval during 10 min. Calibration curves for Furosemide and Rizatriptan were obtained using the mixtures of the drugs with different concentrations in

optimum pH values and using degradation values as responses. For analysis of drugs in real samples, a series of the solutions with known concentrations of Furosemide and Rizatriptan in their calibration range were mixed with 200 µl of prepared serum samples and were analyzed after pH adjustment in the same manner of the calibration samples.

All recorded kinetic-spectrophotometric data matrices were transferred to MATLAB and augmented in the proper direction for MCR-ALS analysis and resolution of the kinetic profiles in order to analyte determination in mixture or real sample.

RESULTS AND DISCUSSION

Accurate determination of the analytes in the mixture by spectrophotometry is highly dependent to the extent of spectral overlapping between components. In the presence of high overlapping, appropriate methodologies like multivariate calibration methods in combination with efficient analytical procedures producing higher amounts of information may be useful to overcome the challenges. In Fig. 2, high overlapping between the components is observed in the pure spectra of Furosemide and Rizatriptan. However, there is a selective region in absorbance spectra of Furosemide with a peak near 330 nm which can be exploited for obtaining selectivity and quantitative determination.

pH of the solution may have a crucial effect on the efficiency of any reaction. This effect may be due to the change in the molecular structure of the analyzed compounds by protonation or deprotonation which will induce electrostatic attraction or repulsion between reactants or participation of the proton and hydroxyl ions in the reaction pathway which leads to pH-dependency of the reaction kinetics. It is widely accepted that the degradation reaction of organic substances by hypochlorite is pH dependent due to the role of the hydroxyl ions in the neutral or basic media [39-41]. Hence, pH optimization is necessary in order to achieve maximum efficiency and differentiate between reaction rates. Optimization experiments were performed in the pH ranges 3-9 in the buffered solution, and as depicted in Fig. 3 a dramatic decrease in degradation kinetics of both drugs were observed above the pH 5. This effect may be caused by electrostatic repulsion between negatively charged drug molecules and hypochlorite anions at more basic medium which inhibits the oxidation reaction to take place. So pH 5 was selected for drug degradation study throughout the experiments. Also to minimize the effect of the oxidant concentration on the reaction rates, excess amounts of hypochlorite were added to initialize the degradation.

Figure 4 shows the normalized degradation profiles of the investigated drugs at the optimum pH. It is evident that there is a slight difference between the kinetic profiles of drugs which may lead to rank-deficient data and subsequent problems in accurate quantification. So, a simple kinetic-spectrophotometric degradation process in combination with chemometrics have been employed using hypochlorite ion as an oxidizing agent to promote the degradation reaction of drugs monitored by spectrophotometry.

As the purpose of this study is the simultaneous determination of Furosemide and Rizatriptan in real samples, the calibration step should be performed in the presence of both analytes. A series of the mixtures containing different concentrations of both Furosemide and Rizatriptan were prepared, and after setting the optimal reaction conditions, kinetic-spectrophotometric data were collected for each mixture. The collected data matrices were augmented in concentration (time) direction in order to break the rank-deficiency caused by the similarities of the kinetic profiles resulting in a multiset data. SVD-based rank analysis showed three meaningful components in augmented calibration samples; two of them are related to drugs and the third component can be attributed to the degradation products. MCR-ALS was performed on the data using pure spectra of the analytes as their initial estimates (equality constraint), non-negativity constraint on both spectral and concentration (time) direction, and unimodality only on concentrations, in addition to the trilinearity constraint to reduce the rotational ambiguity. The initial estimates for degradation products were obtained by implementation of SIMPLISMA [42] on the multiset data. Calibration curves and related parameters were calculated using resolved kinetic profiles of each binary sample. Calibration curves were linear in the ranges 3-50 µM and 2-50 µM for Furosemide and Rizatriptan, respectively. Calibration equations were $A = 23104c + 0.0272$ for

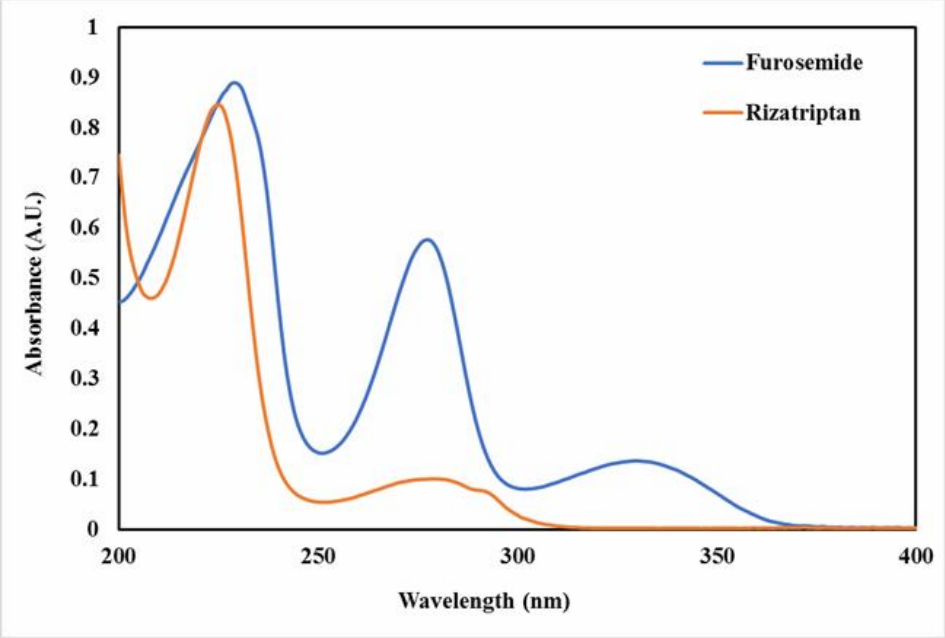

Fig. 2. UV spectra of Furosemide and Rizatriptan.

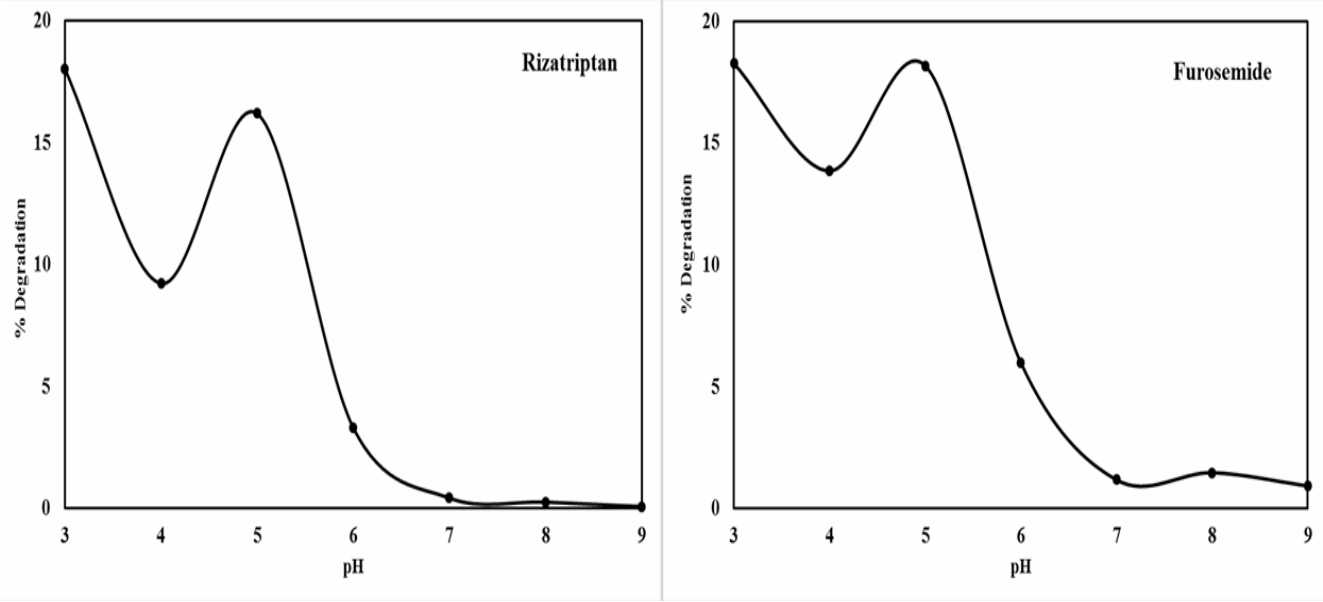

Fig. 3. Effect of pH on the degradation of Furosemide and Rizatriptan.

Furosemide (R2 = 0.998) and A = 29074c + 0.0141 for Rizatriptan (R2 = 0.999), where A is the absorbance in arbitrary units and c is the molar concentration. The values for limit of detection (LOD) of the method were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3. Based on the standard deviation of the

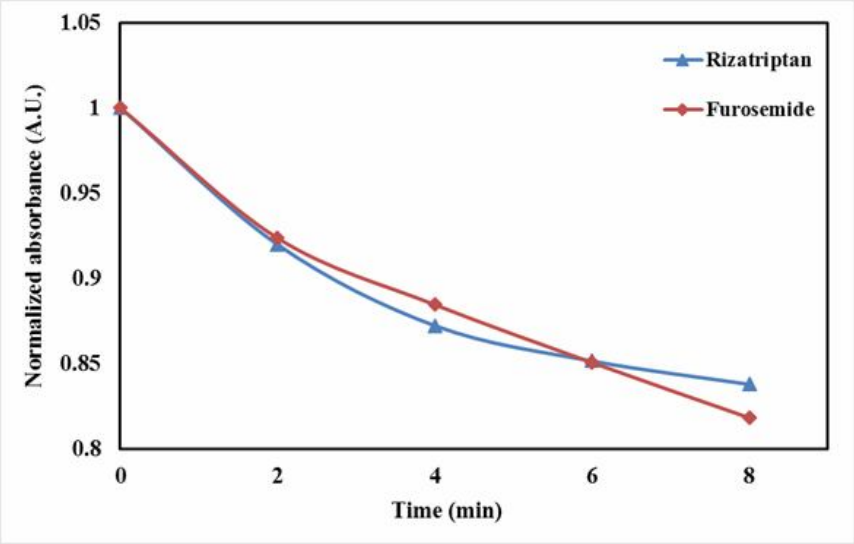

Fig. 4. Normalized degradation profiles of Furosemide and Rizatriptan.

Table 1. Recovery Results in Spiked Serum Samples

Sample No.	Furosemide			Rizatriptan		
	Added (μM)	Found (μM)	Recovery (%)	Added (μM)	Found (μM)	Recovery (%)
1	5	5.1	102	14	13.2	94.3
2	7	7.4	105.7	19	18.9	99.5
3	16	16	100	2	1.87	93.5

repetitive blank measurements LOD was calculated as 0.72 μM and 0.66 μM for Furosemide and Rizatriptan, respectively.

In order to evaluate the applicability of the proposed method for drug determination in real samples, recovery experiments were performed using spiked serum samples as they were obtained from healthy volunteers who did not use drugs before. 200 μl of the prepared serum samples were spiked with the known amounts of analytes, and the kinetic-spectrophotometric data were provided for each solution. One of the effective methods for reducing or even eliminating the rotational ambiguity in MCR methods for accurate quantification is the use of selectivity constraint [43-45] by augmenting a series of data matrices which represent the contribution of only one of the system constituents in the studied phenomenon or indeed data matrices for pure components. In this case, the appearance of zero values in the known positions of resolved profiles, where other components are absent, dramatically reduces the rotational ambiguity intrinsically associated with the MCR techniques.

In this manner, for accurate determination of analytes in the spiked real samples containing unknown interferences, a series of matrices related to the degradation of pure

Furosemide or pure Rizatriptan were augmented alongside the matrix of the real sample in the concentration direction. SVD-based rank analysis showed four or five meaningful components in the case of real samples in augmented data; two of them are related to the studied analytes and the other can be attributed to the degradation products and serum constituents. The resulting multiset data were analyzed by MCR-ALS using non-negativity constraint on both spectral and concentration direction, unimodality only on concentrations and trilinearity. Pure spectra of Furosemide and Rizatriptan were used as initial estimates for drugs while SIMPLISMA was used for obtaining initial estimates for other interfering components. Selectivity constraint was applied as vectors describing absence (zero values) or presence (NaN values) of a specific component in a submatrix. Table 1 shows the recovery results for three different spiked serum samples analyzed by the proposed method.

Satisfactory recovery results for Furosemide and Rizatriptan indicate the suitability of the proposed method for determination of investigated pharmaceutical compounds in routine analysis of real samples. The advantages of the proposed method are its simplicity, speed, cost-effectiveness and independence from sample preparation steps. Combination of the kinetic-spectrophotometric methods capable to produce multivariate bilinear data with efficient and versatile chemometric techniques such as MCR-ALS with a wide variety of applicable constraints, provides the opportunity for the fast and accurate determinations in complex mixtures with highly overlapped spectra or similar kinetic profiles which may give incorrect results using other analytical techniques because of the rank-deficiency or even need to expensive instrumentation or time-consuming preprocessing steps.

CONCLUSIONS

An efficient methodology for the fast and reliable simultaneous determination of Furosemide and Rizatriptan in mixtures and real samples is proposed based on the chemometric analysis of kinetic-spectrophotometric multiset data obtained by the simultaneous degradation of analytes by hypochlorite at optimized pH conditions which shows high spectral overlapping besides similar reaction rates which result in serious rank-deficiency problems. Using MCR-ALS on the multiset data by the implementation of multiple logical constraints helps break the rank-deficiency and obtain accurate results. Recovery experiments in blood serum samples were performed to evaluate the accuracy of the proposed method and satisfactory results were obtained. The proposed method has some advantages over other conventional methods like speed, simplicity, and cost-effectiveness. Additionally, this technique does not require expensive instrumentation or complex sample preparation procedures. These advantages make the proposed method promising for routine analyte determinations.

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