Ultra Performance Liquid Chromatography for the Quantitative Analysis of Ampicillin Sodium and Sulbactam Sodium in a Pharmaceutical Preparation

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In this article a new method based on ultra performance liquid chromatography (UPLC) was proposed for the simultaneous rapid quantitative analysis of of ampicillyn sodium (AS) and sulbactam sodium (SS) in a commercial preparation. Chromatographic separation and quantitation of allura red were performed on an Acquiting UPLC™ BEH Phenyl (100 mm x 1.0 mm, i.d., 1.7 μm) column system and a mobile phase corresponding to a mixture cosisting of acetonitrile and 0.1 M $(NH_4)_2CO_3$ (70:30, v/v) containing 2 mL of trietylamine at a constant flow rate, 1.0 mL/min. Chromatographic calibration graphs in the linear range of 10-80 μg/mL for SS and 10-70 μg/mL for AS was calculated by using the linear regression analysis based on the relationship between the concentration and the peak area with a detection at the wavelength 220 nm. The UPLC method was validated by using the synthetic mixtures and successfully applied to the UPLC quantitation of the commercial samples of the related drugs. We concluded that a good agreement of UPLC quantitation results was reported.

Keywords: Ultra performance liquid chromatography, ampicillin sodium, sulbactam sodium

The combination of sulbactam (SS) and ampicillin (AS) in the pharmaceutical preparations is used in antibacterial therapy for several years. In addition the SS denotes a competitive and irreversible beta-lactamase inhibitor. Synergy with beta-lactam antibiotics is most marked in bacterial species in which beta-lactamase represents a major mechanism of resistance. Polymerizatýon of Penicillins 5. separation, identifýcation and quantitative analysis of antigenic polymerization products in ampicillin-sodium preparations were investigated by high-performance liquid-chromatography [1]. A high-performance liquid chromatographic method using precolumn derivatization and fluorescence detection was proposed and validated for the determination of AS in serum [2]. An accurate and reproducible method for the simultaneous determination of AS, SS and cefoperazone in pharmaceutical formulations by making use of HPLC with P-CD stationary phase was presented in [3]. Also, the simultaneous determination of AS and SS in their binary combination formulation was investigated by spectrophotometry [4], capillary electrophoresis and HPLC [5], by fractional wavelet approach [6] and by wavelet method [7].

HPLC has been used widely in the chemical and pharmaceutical analysis but it requires a longer analysis time and may not give better results for some of sample analysis in some cases. In recent years the UPLC started to be considered as the development of chromatographic performance and chromatographic Software. Nowadays this new UPLC technique performs separations using smaller columns packed with smaller particles (1.7 μm). In addition to that, at higher flow rates UPLC provides accurate and precsise results in a shorter analysis time with superior peak capacity and sensitivity.

The main purpose of this manuscript is to developed a new UPLC method for the analysis of SS and AS in commercial samples. In this study a UPLC H–class instrument with PDA detector system was used for the chromotographic eluation of the related compounds in the short time period of analysis.

The validation of the developed UPLC method was performed by analyzing different binary mixture systems of AS and SS. The stadard addition technique was used for the proposed UPLC method validation. We observed that the developed new UPLC approach can be used for the quality control of the analyzed drugs in samples.

Experimental part

Apparatus

UPLC system based on the use of Acquity UPLCTM BEH C18 column (50 mm x 2.1 mm, 1.μ7 (m i.d.) was used for the chromatographic analysis. Detection responses were recorded in terms of peak area at the wavelength 220 nm. The Acquity UPLC software had the integration of the peak areas. Linear regression analysis and statistical treatments were performed by Microsoft Excel.

Chemicals

Methanol was of HPLC grade (Merck, Germany). $(NH_4)_2CO_3$ (Merck, Germany) and triethylamine were of guaranteed reagent grade. Chromatographic double distilled water was used during chromatographic analysis. AS and SS were kindly donated from a Turkish Pharm.Ind. Company.

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Standard Solutions

Stock solution (25 mg/50 mL) of AS and SS were prepared in mobile phase. Standard series of AS and SS in the concentration range of 10-80g/mL was obtained by using the above stock solution. All of the calibration and sample solutions were prepared within the mobile phase during this study. The standard addition technique was applied to the samples obtained by adding the stadard solutions of AS and SS to the commercial samples.

Chromatographic conditions

UPLC separation and analysis was carried out by using a mobile phase consisting of methanol and 0.1 M $(NH_4)_2CO_3$ (70:30, v/v) with 2% triethylamine and Acquity $UPLC_{TM}$ BEH C18 (50 mm x 2.1 mm, i.d., 1.7 μm) column system. This separation was performed at a constant flow rate and 1.0 mL/min. Column temperature was maintained at 75°C. Colum pressure was observed as 470 bar under above chromatographic conditions. All solvents were filtered through a 0.22 mm Millipore filter. 1.0 μL for all samples was used as injection volume. Quantification was performed by measuring at 220 nm.

Results and discussions

The quantitative analysis of AS and SS in commercial samples for injection was carried out by UPLC method. The classical spectrophotometric determination of AS and SS in commercial samples is not possible due to overlapping spectra or the presence of samples matrix. Therefore, the analysis requires the use of a chromatographic column separation. In the UPLC analysis, various UPLC conditions were tested to find the optimal chromatographic ones. By using an Acquity $UPLC_{TM}$ BEH C18 (50 mm x 2.1 mm, 1.7 μm i.d.) column, the mobile phase containing methanol and $(NH_4)_2CO_3$ (70:30, v/v) containing 2% trietylamine and a constant flow rate at 1.0 μL/min were found to be optimal ones for separation and determination of AS and SS in samples. At constant column temperature 75°C, this UPLC separation is based on an isocratic elution for the analysis of the subjected drugs. Chromatograms were obtained by using PDA detector at the wavelength, 210 nm. Chromatographic detector outputs were measured as peak areas. In the chromatographic analysis, 1.0 μL of the sample solutions were injected into the UPLC column system and injections for each sample analysis were repeated three times. Under the above mentioned UPLC settings, the retention time for AS and SS were observed to be 1.465 min and 0.730 min, respectively as shown in figure 1.

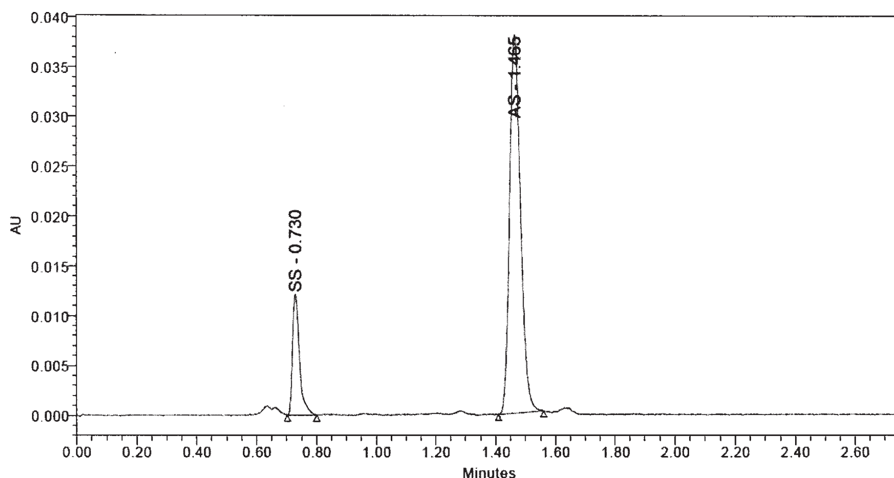
Calibration solutions for both AS and SS in the concentration range of 10.0-80.0 μg/mL were prepared by using the mobile phase. After that the chromatograms of these calibration solutions were registered. A UPLC chromatogram of the analyzed AS and SS compounds were shown in figure 1. Calibration equations and statistical results were obtained by calculating the relationship between concentration and UPLC peak areas. Linear regression analysis and its statistical results were summarized in table 1. The evaluation of the amount of AS and SS in samples was performed within the linear regression equations.

UPLC Method validation

For the quantitative analysis of AS and SS at 210 nm, the linearity of UPLC detector response was observed in the working concentration range of 10.0-80.0 μg/mL at the eight different concentration levels. UPLC analysis for each concentration level was repeated three times to get better information on the variation in peak area between samples. The linearity for the calibration equations of AS and SS was reported by the high correlation coefficient values (table 1). A linear regression analysis and results were represented in table 1.

Proposed UPLC method was validated by analyzing 16 mixtures of the related drugs. Recovery data and standard deviation with relative standard deviations were given in table 2. These numerical values indicate a good accuracy for the proposed UPLC methods. The precision and accuracy of the developed UPLC procedure was tested by using the standard addition samples at different concentration levels within the working concentration range. Recoveries and the relative standard deviations were found as 97 and 2.17% for SS and 96.9 and 1.42 for AS.

A graph between peak areas and the concentrations of standard addition samples for SS and AS was obtained as it is shown in figures 2A and 2B, respectively. In these graphics, 7.31 μg/mL (expected value =7.5μg/mL) for SS and 14.55 μg/mL (expected value =15.0 μg/mL) for AS were obtained from the absolute intercept values on the abscise axis. In addition the slopes of the linear regression equations of the calibrations solution and standard additions samples are closed to each other (figs. 2A-B and table 1). This result indicates that any interference wasn't observed during the UPLC analysis. A good coincidence between the analysis results was reported for the developed UPLC method. The limit of detection (LOD) and quantization (LOQ) were calculated by using the standard deviation of the response and the slope of the linear regression line (table 1).



Fig, 1. UPLC chromotagram of 30 μg/mL SS and 60 μg/mL AS compounds

Table 1
LINEAR REGRESSION ANALYSIS AND STATISTICAL RESULTS

Parameter	SS	AS
λ (nm)	210	210
Range (μg/mL)	10-80	10-80
m	791.02	2192.85
n	-15.25	2196.86
r	0.9999	0.9999
SH(m)	4.41	13.32
SH(n)	222.65	672.67
SH(r)	285.75	863.29
LOD	2.39	2.60
LOQ	7.96	8.68

m = Slope of the linear regression equation
n = Intercept of the linear regression equation
r = Correlation coefficient of the linear regression equation
SE(m) = Standard error of the slope
SE(n) = Standard error of the intercept
SE(r) = Standard error of the correlation coefficient
LOD = Limit of detection
LOQ = Limit of quantitation

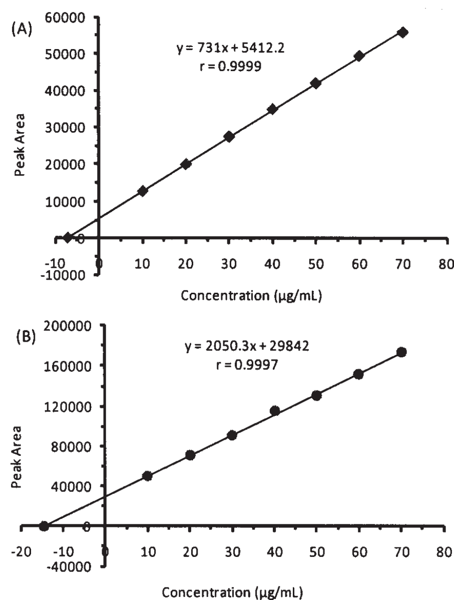


Fig. 2. Graphs for the standard addition samples of SS (A) and AS (B)

Table 2
RECOVERY RESULTS OF THE SS AND AS COMPOUNDS IN THEIR BINARY SAMPLES BY THE DEVELOPED UPLC METHOD

	Mixture (μg/mL)		Found (μg/mL)		Recovery (%)	
No.	SS	AS	SS	AS	SS	AS
1	30	10	31.14	10.18	103.8	101.8
2	30	20	29.68	19.68	98.9	98.4
3	30	30	29.99	30.38	100.0	101.3
4	30	40	29.20	39.01	97.3	97.5
5	30	50	31.79	49.63	106.0	99.3
6	30	60	31.51	57.04	105.0	95.1
7	30	70	31.45	71.84	104.8	102.6
8	30	80	31.19	80.98	104.0	101.2
9	10	60	10.24	59.89	102.4	99.8
10	20	60	20.81	60.88	104.1	101.5
11	30	60	31.08	58.32	103.6	97.2
12	40	60	41.17	59.21	102.9	98.7
13	50	60	49.90	59.78	99.8	99.6
14	60	60	58.98	60.59	98.3	101.0
15	70	60	69.66	61.77	99.5	102.9
16	80	60	75.06	59.24	93.8	98.7
				mean	101.5	99.8
				SS	3.38	2.15
				RSD	3.33	2.15

SD = Standard deviation, RSD = Relative standard deviation

Table 3
DETERMINATION RESULTS FOR THE COMMERCIAL FORMULATION SAMPLES BY THE DEVELOPED UPLC METHOD

	mg/tablet	
No.	SS	AS
1	0.49	1.02
2	0.51	1.06
3	0.47	1.01
4	0.50	1.02
5	0.53	1.01
mean	0.5	1.0
SS	0.02	0.02
RSD	4.20	2.26

SD = Standard deviation, RSD = Relative standard deviation

Sample analysis

The developed UPLC was directly applied to the quantitative analysis of allura red in a commercial samples. 1.0 μL of the commercial samples were injected into UPLC column system and then the chromatogram was obtained by using the detection at 220 nm. The determination results of five repetition assays were shown in tables 3. Their mean values, standard deviation (SD), relative standard deviation (RSD) were presented in the above table.

Conclusions

The method validation data indicates that the developed UPLC method is reliable, reproducible and accurate for the determination of AS and SS in samples. The proposed rapid UPLC method is a powerful new technique for the chromatographic separation and determination of AS and SS in all samples. We conclude that the UPLC approach is suitable for routine assessment of the quality of commercial samples.

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