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Simultaneous Determination of Ascorbic Acid, Paracetamol, Aspirin in Tablets Using UPLC

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Abstract

Ultra-high-performance liquid chromatographic (UPLC) data obtained from photodiode array (PDA) detection was processed by the PCR and PLS algorithms for the simultaneous quantitative resolution of Ascorbic Acid (AA), Paracetamol (PAR), Aspirin (ASP) in a commercial formulation. Principle component regression (PCR) and partial least squares (PLS) were applied to the peak area ratio of the analytes/internal standard at multi-wavelength PDA detector responses. The combined use of UPLC and chemometric calibration techniques was denoted UPLC-PCR and UPLC-PLS. For the comparison purpose, the UPLC method was used for the confirmation of the results obtained from combined UPLC-chemometric calibration techniques. A good chromatographic separation between drugs and internal standard (IS) was achieved using a Waters ACQUITY UPLC BEH Phenyl (100 mm x 1.0 mm, i.d., 1.7 µm) column and a mobile phase consisting of 0.1 M CH_3COOH and methanol (75:25, v/v).

The multi-wavelength PDA detection for Ascorbic Acid (AA), Paracetamol (PAR), Aspirin (ASP) was accomplished by measuring the peak area at the wavelength set corresponding to 245, 250, 255.0, 260.0, 265, 270, 275 and 280 nm. The proposed UPLC-PCR and UPLC-PLS approaches were validated by using the synthetic mixtures, and inter-day and intra-day experiments. The proposed methods were successfully applied to commercial samples containing the analyzed drugs.

Keywords: Quantitative resolution, UPLC method, Paracetamol, Ascorbic acid, Aspirin; Pharmaceutical preparation

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Özet

Tabletlerde Askorbik Asit, Parasetamol ve Aspirinin UPLC Yöntemi ile Eşzamanlı Analizi

Ticari tablet formülasyonu içerisindeki Askorbik Asit (AA), Parasetamol (PAR) ve Aspirinin (ASP)'nin kantitatif eş zamanlı miktar tayinleri için fotodiod dizili dedeksiyonu (PDA) kullanılarak elde edilen aşırı yüksek perfomranslı sıvı kromatografik (UPLC) verileri, kısmi en küçük kareler (PLS) ve temel bileşen regresyon (PCR) algoritmaları ile proses edildi. Kısmi en küçük kareler ve temel bileşen regresyon algoritmaları, çok dalgaboyunda PDA detektör cevaplarından elde edilen analit/iç standart pik alanları oranına uygulandı. UPLC ve kemometrik kalibrasyon tekniklerinin kombine kullanımı, UPLC-PCR ve UPLC-PLS olarak adlandırıldı. Karşılaştırma amacıyla, klasik UPLC yöntemi, kombine UPLC kemometrik tekniklerden elde edilen sonuçları doğrulamak için kullanıldı.

Analiz edilen ilaçlar ve iç standart madde arasında iyi bir kromatografik ayrımı, Waters ACQUITY UPLC BEH fenil (100 mm x 1.0 mm, i.d., 1.7 µm) kolon ile 0.1 M CH_3COOH ve metanol (75:25, v/v) kullanılarak gerçekleşitrildi. Çalışmada Askorbik Asit (AA), Parasetamol (PAR) ve Aspirinin (ASP)'nin çoklu dalga boylarındaki PDA ölçümleri 245, 250, 255.0, 260.0, 265, 270, 275 ve 280 nm dalgaboylarına karşılık gelen dalga boylarının pik alanlarının ölçümü ile elde edildi. KAF'ın internal standart olarak varlığında, Askorbik Asit (AA), Parasetamol (PAR) ve Aspirinin (ASP)'nin sentetik karışımları ile gün içi ve günler arası numunelerin analizleriyle UPLC-PCR ve UPLC-PLC yöntemlerinin validasyonu gerçekleştirildi. Bu çalışmada önerilen yöntemlerin analizi edilen ilaçları içeren ticari örnekleri analizlerine başarıyla uygulandı.

Anahtar Kelimeler: Kantitatif çözümleme, UPLC metodu, Parasetamol, Askorbik asid, Aspirin; Pharmasötik Hazırlık.

Introduction

Paracetamol (also called acetaminophen) is a widely used as analgesic and antipyretic agent. The main mechanism proposed is the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2 [1]. The analgesic activity is attributable to the small fraction that penetrates into the brain. The analgesic mechanism of paracetamol, being that the metabolites of paracetamol e.g. NAPQI, act on TRPA1-receptors in the spinal cord to suppress the signal transduction from the superficial layers of the dorsal horn, to alleviate pain [2]. The COX family of enzymes are responsible for the metabolism of arachidonic acid to prostaglandin H_2, an unstable molecule that is, in turn, converted to numerous other pro-inflammatory compounds. Only when appropriately oxidized is the COX enzyme highly active [3]. Paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals [4]. Ascorbic acid is an antioxidant, along with vitamins. Vitamin C is an electron donor, and this property accounts for all its known functions. Antioxidants block some of the damage caused by free radicals, substances that damage DNA [5]. Vitamin C is essential for the development and maintenance of connective tissues [6]. Aspirin is used in the treatment of mild to moderate pain, inflammation, and fever. It is also used as an antiplatelet agent to prevent myocardial infarction, stroke and transient ischemic episodes. Aspirin's ability to suppress

the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase COX [7]. A combination of paracetamol, acetylsalicylic acid, ascorbic acid is widely used against inflammation in the body, fever and reduction of biological factors that cause pain. Same combination has also analgesic and antipyretic effects.

In previous studies, several methods including spectrophotometric [8-9], chromatographic [10-11], electrochemical [12] and signal processing-chemometric PCR and PLS [13] methods were reported for the simultaneous quantitative analysis of AA, PAR and ASP. Analysis of binary mixtures of ASP and PAR were carried out by spectrophotometry [14], HPLC [15-16] and voltammetry [17-18]. Simultaneous determination of ASP and AA were performed by spectrophotometric chromatographic [20-21] and electrochemical [22] methods. Chromatographic [23-24], electrochemical and spectrophotometric [25] determination studies were conducted to analyze AA and PAR in their binary mixtures.

HPLC or UPLC is the most commonly used method for the analysis of drugs in pharmaceutical formulations. In practice, the chromatographic methods with chemometrics have been used to get more accurate, precise and reliable analysis of active compounds, and to resolve the co-elution of peaks in a chromatogram.

In chromatography, classical UPLC gives to rise some detection errors of peak area come from injection, instrumental fluctuations and other sources in case of a single wavelength detector response.

On the other hand, the simultaneous use of chromatograms obtained at multi-wavelength PDA detector response will allow to eliminate the errors of single regression equation based on single wavelength detection. Classical UPLS method requires a specified detection wavelength for each analyte in a multi-component mixture. In contrast to this conventional UPLC technique, UPLC-multivariate calibration techniques (PCR and PLC) based on the multi-wavelength set doesn't require one specified detection wavelength that cause some calibration errors. In addition, the use of UPLC-chemometric calibrations instead of single wavelength detection-UPLC technique provides the higher precision and accuracy for the analysis results.

Nowadays, chemometric calibrations such as principle component regression (PCR) and partial least squares (PLS) has been used to the analysis of the chromatographic data for the multi-component analysis [26-27] in order to overcome drawbacks coming from classical UPLC analyses.

In this study, PCR and PLS calibration techniques were proposed for the UPLC data set at the multi-wavelengths set for the analysis of the ternary mixture containing three compounds, AA, PAR and ASP. These PCR and PLS methods combined with UPLC were named as UPLC-PCR and UPLC-PLS during this article. After the method validation procedure, the proposed UPLC-PCR and UPLC-PLS were applied to the analysis of the commercial preparation containing PAR, AA, ASP. As an alternative approach, a classical UPLC was developed for the analysis of the related drugs in samples. A good agreement was reported for the assay results to get successful determination of the analyzed compounds.

Materials and Methods

In this study, PCR and PLS algorithms are implemented in the ratio of the peaks area of analyzed drugs to IS at the eight wavelengths using a PDA detector. The detector responses were measured in terms of peak area. The application procedure of the combined UPLC-chemometrics calibrations was described below for each chemometric calibration.

UPLC-PCR Method

In the UPLC-PCR application, ordinary PCR algorithm was used. In the PCR application, the ratio (R) of the peak area of individual drug and the drug concentration set (C) were reprocessed by mean-centering as R_o and C_o, respectively. The covariance dispersion matrix of the centered matrix R_o was calculated.

The normalized eigenvalues and eigenvectors were obtained from square covariance matrix. The number of the optimal principal components (eigenvectors (P)) is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated. In order to reach this objective, the coefficient b defined as $b = P \times q$ is determined, where P is the matrix of eigenvectors and q is the C–loadings given by $q = D \times T^T \times R_o$. T^T is the transpose of the score matrix T and D is a diagonal matrix having the components the inverse of the selected eigenvalues. The drug content in samples was predicted by using the $C_{prediction} = b \times R_{sample}$. In this study PLS toolbox 3.0 in Matlab 7.0 software was used for the data treatment.

UPLC-PLS Method

The PLS calibration using the orthogonalized PLS algorithm developed by World [28-29] and extensively discussed by Martens and Naes [30] involves simultaneously the independent and the dependent variables on the data compression and decomposition operations. In the PLS algorithm, the UPLC-PLS calibration is done by decomposition of both concentration and the ratio of peak area matrix into latent variables, $R=TxP^T+ E$ and $C = U \times Q^T + F$. The linear regression, $C_{prediction} = b \times R_{sample}$, is used for the estimation of the drugs in the samples. The vector, b is given as $b = W \times (P^T \times W)^{-1} \times Q$, where W is a weight matrix [31]. Calibration and prediction steps were done by means of PLS toolbox 3.0 in Matlab 7.0 software.

Instrumentation and Software

Chromatographic analyses were performed with an WATERS® Acquity H-Class UPLC system a thermostatted autosampler, a thermostatted column compartment, and WATERS® Acquity H-Class UPLC ile «photo diode array» detector (DAD). Chromatographic data were acquired and processed by E-power software (Waters, USA) WATERS® Acquity H-Class UPLC using. A Waters Acquity UPLC™ BEH Phenil colonum (100 mm x 1.0 mm, i.d., 1.7 µm) was used for the separation and analysis of active compounds.

The flow rate was maintained at 0.35 mL/min and the injection volume was 3,5 µL. The mobile phase was prepared daily, filtered through a 0.20 µm membrane filter.

Commercial Tablet Formulation

The commercial pharmaceutical formulation (Afebryl Effervescent Tablet Smb Technology S.A. Belgium Galepharma) containing Paracetamol 200 mg acetylsalicylic acid 300 mg ascorbic acid 300 mg were analyzed by using the proposed UPLC-PCR and UPLC-PLS methods.

Standard Solutions

Stock solution of 20 µg /mL PAR, AA, ASP and CAF were individually prepared in mixture of 0.1 M acetic acid and methanol (v/v, 75:25). A concentration set containing 5.0-30.0 µg / mL PAR, AA, ASP was obtained from the stock solutions. A validation sample consisting of 19 synthetic mixture solutions in the same range of 5.0–30.0 µg /mL PAR, AA, ASP was prepared. For standard addition method, six solutions using the stock solutions and tablet solutions were prepared. In all the chromatographic analyses, 15.0 µg /mL caffeine as internal standard (IS) was added into each solution.

Tablet Analysis

In the analyses of effervescent tablets, 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in the mobile phase in a 100 ml calibrated flask. The sample solution was sonicated and filtered into a 100 ml calibrated flask by a 0.45 μm membrane filter. Resulting solution was diluted with mobile phase system 15μ of this sample solution was injected into UPLC system for the analysis of three drugs AA, PAR and ASP in samples.

Results and Discussion

The use of multi-wavelength detections provides different peak area at the multi-wavelength set. Simultaneous data collection at multi-wavelengths allows to apply the chemometric calibration technique to these UPLC data for the qualitative and quantitative analyses. The multivariate method application to UPLC data requires the collection of peak area at multi-wavelengths. In this study, the UPLC-PCR and UPLC-PLS methods were improved for the simultaneous quantitative resolution of ternary system containing, AA, PAR and ASP using multi-wavelength detection. In addition, the newly classical UPLC method was developed for the comparison of the analysis results obtained from UPLC-PCR and UPLC-PLS methods.

The UPLC data set of the peak area ratio for the data treatments of the UPLC-chemometric calibrations were listed in Table 1. These data set were used to construct the UPLC-PCR and UPLC-PLS calibrations.

In this table, the peak area ratio was taken for each drug peak area to IS peak area. The UPLC-chemometric calibrations constructed and were used for the estimation of the amounts of drugs in samples. Then the analysis results provided UPLC-chemometric calibration methods were compared with those obtained by classical-UPLC method.

In this study, the fundamental purpose of multivariate calibration techniques to the multivariate UPLC dataset is the elimination of the errors coming from sample injection and experimental environment that affect the peak area. Therefore, UPLC-chemometric calibration permits to remove the errors and residuals of calibration of the classical UPLC based on a single wavelength. This study indicated that Sensitivity, accuracy and precision of the UPLC-chemometric calibrations increased in comparison with the classical-UPLC method. (See Table7). The application of the multivariate calibration algorithms to the analysis of the related drugs described below.

UPLC data procedure

The concentration set of the mixture solutions containing, AA, PAR, ASP in the concentration range of 5.0–30.0 μg /mL in the presence of 15.0 μg /mL IS was prepared. The peak area of concentration set was recorded at a eight-wavelength set (245, 250, 255, 260, 265, 270, 275 and 280 nm) and at the retention time of 0.552 for PAR, 0.354 for AA, 1.802 and 0.892 for IS. The chromatograms of concentration set in the concentration range for three drugs were illustrated in Figure 1. The chemometric calibration techniques, PCR, and PLS were subjected to the concentration set and peak area-ratio dataset to computed UPLC-PCR and UPLC-PLS models. The concentration of PAR, AA and ASP in samples were determined by using the UPLC-chemometric calibrations

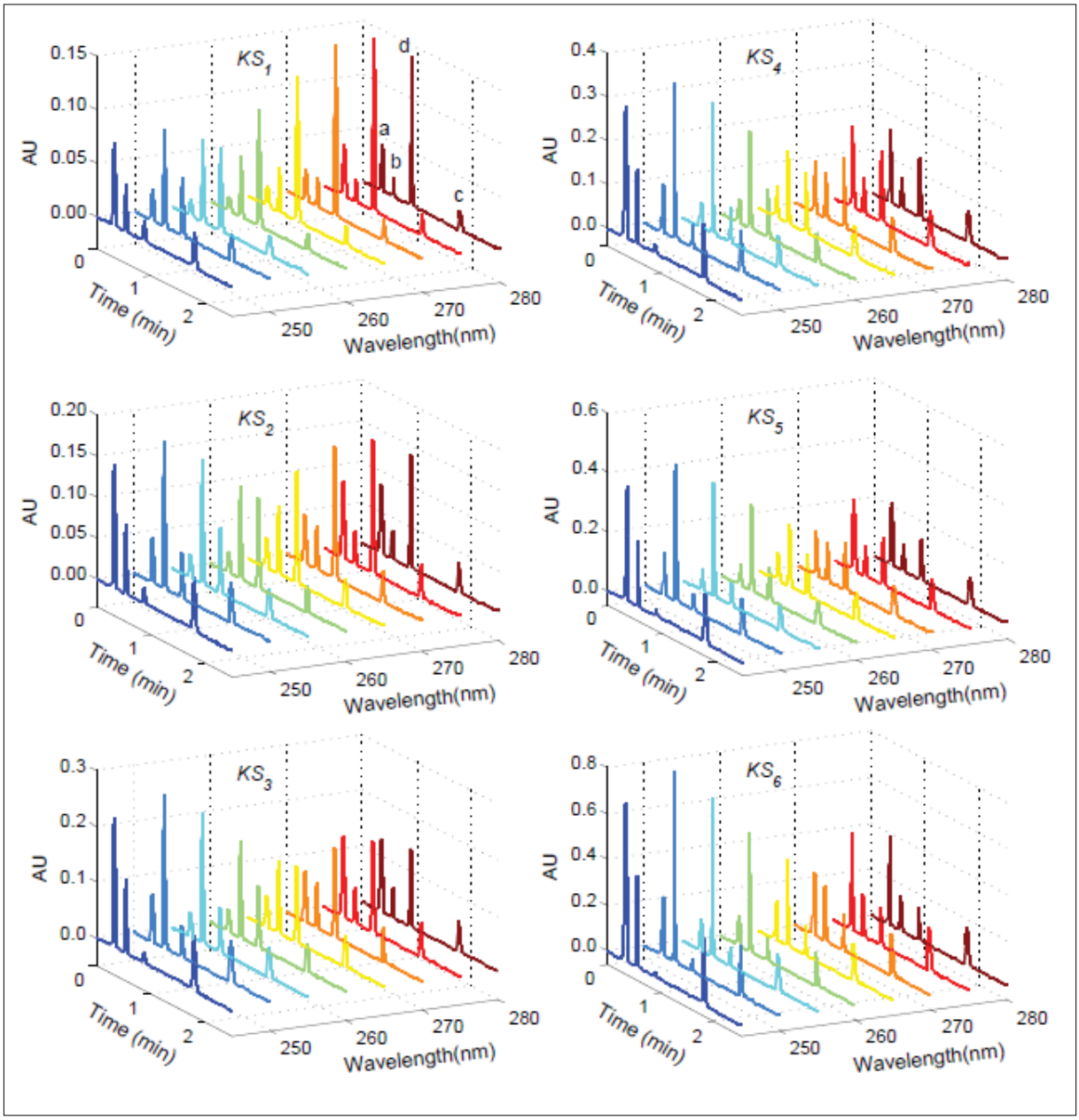

Figure 1. 3 Dimensional UPLC chromatograms of the calibration set at the multi-wavelength set from 245 nm to 280 nm with the interval of Δλ= 5.0 nm

3.2 UPLC-PCR Method

The UPLC-PCR calibration was obtained by using the PCR algorithm. In this calibration, the square matrix of peak area data was obtained by decomposing peak area values. The PCR algorithmic relationship between concentration set and decomposed peak area values was used to get the UPLC-PCR calibration.

This algorithmic process was separately repeated for each drug substance. The calculated UPLC-PCR calibration was applied to the analysis of the related drugs in the synthetic mixtures and tablet samples. The dataset, which was given in Table 1, calculated from the chromatograms corresponding to Figure 1 was used for the computation of UPLC-PCR calibration The calculation of calibration and data treatment were carried out by using the PLS toolbox 3.0 in Matlab 7.0 software.

3.3 UPLC-PLS method

In the application of this method, PLS calibration algorithm was used for the mathematical treatment of the UPLC data summarized in Table 1. The simultaneous decomposition of peak area data and concentration set was performed to get UPLC-PLS. The quantitative analysis of three drugs in samples was performed by the using UPLC-PLS calibration. The mathematical procedures have been accomplished by means of the PLS toolbox 3.0 in Matlab 7.0 software.

In the chemometric calibration study, another important parameter is the standard error of prediction (SEP). The SEP values and their statistical values were calculated according to the difference between added and predicted concentrations in the synthetic mixtures.

The obtained results, SEP, correlation coefficient (r), slope (m) and intercept (n) were presented in Table 2. All the statistical data indicate that the minimum values of SEC and SEP give us acceptable results under optimized conditions in the calibration and prediction steps when first two factors were used for UPLC- PCR and UPLC-PLS

Table 1. The UPLC data set corresponding to the concentration set, drug and IS peak area

				AA, Time (minune) = 0.364							
	AA	***PAR***	***ASP***	***Area/IS***							
		(µg /L)		245	250	255	260	265	270	275	280
1	5	5	5	0.9200	0.8578	0.4978	0.2518	0.1433	0.1148	0.0703	0.0531
2	10	10	10	1.8391	1.6024	0.9743	0.5160	0.3163	0.2215	0.1502	0.1170
3	15	15	15	2.9509	2.3393	1.4106	0.8141	0.4947	0.3243	0.2438	0.1851
4	20	20	20	3.8138	3.1051	1.8874	1.0889	0.6702	0.4321	0.3083	0.2515
5	25	25	25	4.8878	3.7972	2.3663	1.3609	0.8440	0.5374	0.3962	0.3167
6	30	30	30	5.8096	4.5465	2.8266	1.6172	0.9971	0.6399	0.4952	0.3831
				PAR, Time (minute) = 0.552							
				Area/IS							
				245	250	255	260	265	270	275	280
1	5	5	5	1.8347	1.4921	0.8726	0.4780	0.2478	0.1551	0.1028	0.1107
2	10	10	10	3.6531	2.8634	1.6246	0.8859	0.4750	0.2809	0.2141	0.2119
3	15	15	15	5.4177	4.3632	2.5425	1.3154	0.7028	0.4072	0.3092	0.3079
4	20	20	20	7.1124	5.7065	3.2537	1.7290	0.9199	0.5367	0.4099	0.4054
5	25	25	25	9.1119	7.1848	3.9771	2.1111	1.1529	0.6648	0.5110	0.5090
6	30	30	30	10.8775	8.5212	4.7780	2.5495	1.3961	0.8043	0.6389	0.6193
				ASP, Time (minute) =1.802							
				Area/IS							
				245	250	255	260	265	270	275	280
1	5	5	5	0.2297	0.1060	0.0497	0.0328	0.0315	0.0372	0.0312	0.0280
2	10	10	10	0.4363	0.1970	0.0944	0.0627	0.0579	0.0626	0.0550	0.0563
3	15	15	15	0.6417	0.3032	0.1335	0.0917	0.0825	0.0867	0.0840	0.0897
4	20	20	20	0.8467	0.3890	0.1760	0.1195	0.1073	0.1103	0.1077	0.1126
5	25	25	25	1.0322	0.4875	0.2243	0.1487	0.1333	0.1371	0.1337	0.1470
6	30	30	30	1.2748	0.5854	0.2666	0.1804	0.1603	0.1614	0.1607	0.1763

Table 2. Cross-validation and factor selection in UPLC-Chemometric calibrations

	UPLC-PLS			***UPLC-PCR***		
	AA	***PAR***	***ASP***	***AA***	***PAR***	***ASP***
SEP	0.2495	0.1381	0.2681	0.2895	0.1381	0.2602
SEC	0.0331	0.0924	0.1279	0.0381	0.0924	0.1395
PRESS	0.00656	0.0512	0.0982	0.0087	0.0512	0.1677

Classical UPLC method

The chromatograms of AA, PAR, ASP in the concentration range of 5.0–30.0 µg /mL AA, PAR, ASP in the presence of 15.0 µg /mL IS were recorded by using diode array detector at the eight-wavelength set as indicated in Figure 1. The chromatographic detector responses were measured in terms of peak area.

Chromatographic elution of the analyzed active compounds was carried out at the ambient temperature 35ºC on Waters Acquity UPLCTM BEH Phenyl Column (100 mm x 1.0 mm, i.d., 1.7 µm) and mobile phase consisted of 0.1 M CH_3COOH and methanol (v/v, 75:25). The flow rate was set 0.35 mL/min with 3.50 mL as injection volume.

IS as an internal standard is suitable in our case as seen in Figure 1. Especially,, several mobile phase and other chromatographic conditions were tested and then but the above chromatographic conditions were found to be suitable for the separation and determination of AA, PAR and ASP in their mixtures. The same conditions were used for the UPLC-chemometric calibrations. At a flow rate of 0.35 mL/min, retention times for AA, PAR, ASP ve IS were 0.354 for AA, 0.552 for PAR, 1.802 for ASP and 0.892 for IS (Figure 1).

For the calibration, the ratio of peak area of analyte to IS was plotted versus the concentration of AA, PAR and ASP. Table 1 indicates the data of the ratio peak area obtained at the five wavelength set 245, 250, 255, 260, 265, 270, 275 and 280 nm.

In the above wavelength points, five straight lines for each drug were obtained from the UPLC data given in Table 1. Three linear regression equations having the highest regression coefficients at 270, 250 and 265 nm AA, PAR and ASP among the calculated calibrations were selected for the analysis of AA, PAR and ASP respectively. The calculated straight lines and their statistical parameters were presented in Table 3. UPLC approaches based on the detection at 270 nm for AA, 250 nm for and 265 nm for ASP were considered as classical UPLC method for the evaluation of the analyzed active compounds. The correlation coefficients of regression equations were found to be higher than 0.99. At the subject wavelength point, the calibration equations give us good linearity and successful results for AA, PAR and ASP.

Table 3. The calculated straight lines and their statistical parameters

				AA, t (sec)= 0.364				
λ (nm)	*m*	*n*	*r*	*SE(m)*	*SE(n)*	*SE(r)*	*LOD*	*LOQ*
245	0.1969	-0.0755	0.9996	2.72E-03	5.09E-02	5.68E-02	1.8988	6.3292
250	0.1474	0.1287	0.9999	8.15E-04	1.59E-02	1.71E-02	0.7915	2.6384
255	0.0931	0.0308	0.9999	5.54E-04	1.08E-02	1.16E-02	0.8507	2.8358
260	0.0551	-0.0222	0.9998	5.66E-04	1.10E-02	1.18E-02	1.4703	4.9010
265	0.0344	-0.0251	0.9997	3.88E-04	7.56E-03	8.12E-03	1.6123	5.3743
270	0.0210	0.0103	1.0000	6.91E-05	1.34E-03	1.44E-03	0.4698	1.5659
275	0.0166	-0.0130	0.9982	5.01E-04	4.66E-03	1.05E-02	2.0620	6.8734
280	0.0132	-0.0138	1.0000	4.45E-05	8.66E-04	9.30E-04	0.4808	1.6025
				PAR, t (sec)= 0.552				
λ (nm)	*m*	*n*	*r*	*SE(m)*	*SE(n)*	*SE(r)*	*LOD*	*LOQ*
245	0.3616	0.0060	0.9998	3.56E-03	6.94E-02	7.45E-02	1.4100	4.7001
250	0.2826	0.0766	0.9999	2.04E-03	3.97E-02	4.27E-02	1.0332	3.4441
255	0.1560	0.1118	0.9994	2.69E-03	5.24E-02	5.63E-02	2.4707	8.2356
260	0.0826	0.0669	0.9999	6.30E-04	1.23E-02	1.32E-02	1.0917	3.6388
265	0.0457	0.0165	0.9999	3.65E-04	7.10E-03	7.63E-03	1.1429	3.8098
270	0.0259	0.0222	0.9999	2.19E-04	4.26E-03	4.58E-03	1.2102	4.0341
275	0.0210	-0.0029	0.9990	4.62E-04	6.99E-03	9.66E-03	2.4490	8.1632
280	0.0202	0.0076	0.9997	2.39E-04	4.65E-03	5.00E-03	1.6940	5.6467
				ASP, t (sec)= 1.802				
λ (n)	*m*	*n*	*r*	*SE(m)*	*SE(n)*	*SE(r)*	*LOD*	*LOQ*
245	0.0412	0.0217	0.9995	6.25E-04	1.22E-02	1.31E-02	2.1675	7.2249
250	0.0192	0.0093	0.9998	2.04E-04	3.97E-03	4.27E-03	1.5232	5.0774
255	0.0087	0.0057	0.9997	1.14E-04	1.52E-03	2.38E-03	1.2903	4.3010
260	0.0059	0.0036	0.9998	5.20E-05	1.01E-03	1.09E-03	1.2709	4.2364
265	0.0051	0.0062	0.9999	3.04E-05	5.92E-04	6.36E-04	0.8545	2.8485
270	0.0050	0.0125	0.9999	3.92E-05	7.63E-04	8.20E-04	1.1309	3.7697
275	0.0052	0.0046	0.9997	5.87E-05	1.14E-03	1.23E-03	1.6211	5.4036
280	0.0059	-0.0020	0.9992	1.19E-04	2.32E-03	2.49E-03	2.8815	9.6049

Validation of UPLC-Chemometric methods

The validity of UPLC-PCR and UPLC-PLS was performed by analyzing the synthetic mixtures and inter-day and intra-day samples. In the validation experiments, 19 different synthetic mixtures containing PAR, AA and ASP in the concentration range of 5-30 mg/mL in the presence of 15.0 mg/mL IS were prepared and analyzed by the UPLC-PCR and UPLC-PLS methods.

The recovery values and their relative standard deviations of the proposed methods were computed and indicated in Table 4. Their numerical values were found satisfactory for the validity of UPLC-PCR and UPLC-PLS. The reliable accuracy and higher precision in the application of these methods were reported for the analysis of three drugs. During the analysis procedure, interference and systematical errors were not reported.

Table 4. Recovery results in ternary mixture by using UPLC-PCR ve UPLC-PLS

				Found (µg/ml)						*Recovery (%)*					
	Added (µg/ml)			*AA*		*PAR*		*ASP*		*AA*		*PAR*		*ASP*	
No.	*AA*	*PAR*	*ASP*	*UPLC-PLS*	*UPLC-PCR*	*UPLC-PLS*	*UPLC-PCR*	*UPLC-PLS*	*UPLC-PCR*	*UPLC-PLS*	*UPLC-PCR*	*UPLC-PLS*	*UPLC-PCR*	*UPLC-PLS*	*UPLC-PCR*
1	5	10	15	4.98	4.98	10.03	10.15	15.19	15.20	99.7	99.6	100.3	101.5	101.3	101.3
2	10	10	15	9.82	9.81	10.26	10.27	15.00	15.00	98.2	98.1	102.6	102.7	100.0	100.0
3	15	10	15	14.97	14.97	10.11	10.14	14.94	14.94	99.8	99.8	101.1	101.4	99.6	99.8
4	20	10	15	20.09	20.10	9.98	10.08	14.96	14.96	100.4	100.5	99.8	100.8	99.7	99.7
5	25	10	15	25.01	25.01	9.98	10.03	14.86	14.88	100.0	100.1	99.8	100.3	99.0	99.2
6	30	10	15	30.63	30.65	10.02	9.97	15.04	15.06	102.1	102.2	100.2	99.7	100.2	100.4
7	15	5	15	15.09	15.09	5.09	5.10	15.09	15.09	100.6	100.6	101.9	102.1	100.6	100.6
8	15	10	15	14.90	14.90	10.17	10.31	15.04	15.04	99.3	99.3	101.7	103.1	100.3	100.3
9	15	15	15	15.04	15.03	15.25	15.24	15.26	15.24	100.2	100.2	101.7	101.6	101.7	101.6
10	15	20	15	15.18	15.18	19.95	19.97	15.02	15.02	101.2	101.2	99.8	99.9	100.1	100.1
11	15	25	15	14.97	14.97	25.13	25.17	15.24	15.24	99.8	99.8	100.5	100.7	101.6	101.6
12	15	30	15	15.07	15.08	29.91	29.85	15.08	15.08	100.5	100.5	99.7	99.5	100.5	100.6
13	15	10	5	15.34	15.35	10.12	10.19	5.09	5.09	102.3	102.3	101.2	101.9	101.8	101.8
14	15	10	10	15.59	15.60	10.09	10.09	10.28	10.26	103.9	104.0	100.9	100.9	102.8	102.6
15	15	10	15	14.78	14.77	10.06	10.13	15.07	15.11	98.5	98.5	100.6	101.3	100.5	100.7
16	15	10	20	15.00	15.00	9.92	9.93	19.62	19.58	100.0	100.0	99.2	99.3	98.1	97.9
17	15	10	25	15.20	15.21	9.98	9.95	24.98	25.06	101.3	101.4	99.8	99.5	99.9	100.2
18	15	10	30	15.33	15.34	9.99	10.00	29.06	29.12	102.2	102.3	99.9	100.0	96.9	97.1
19	15	10	15	14.87	14.87	9.96	10.02	15.20	15.20	99.1	99.1	99.6	100.2	101.3	101.3
									X	100.5	100.5	100.5	100.9	100.3	100.4
									SS	1.42	1.45	0.94	1.11	1.37	1.33
									BSS	1.42	1.44	0.94	1.10	1.36	1.32

Another parameter for validity of developed approaches is the analysis of the intra-day and inter-day samples. The results and their standard deviations were calculated and presented in Table 5 and 6. The recovery results were obtained in the average of five replicate for each compounds. A good coincidence was reported for the assay results of the intra-day and inter-day experiments by application of the UPLC-PCR and UPLC PLS methods.

Table 5. Analysis results of inter-day by UPLC-PLS and UPLC-PCR

					Found (µg/ml)			
Added (µg/ml)			***AA***		***PAR***		***ASP***	
AA	***PAR***	***ASP***	***PLS***	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
10	10	10	10.11	10.11	10.06	10.07	10.31	10.30
20	20	20	19.96	19.96	19.55	19.54	19.89	19.92
30	30	30	30.90	30.91	29.42	29.39	29.57	29.65
					Recovery (%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			101.1	101.1	100.6	100.7	103.1	103.0
			99.8	99.8	97.8	97.7	99.4	99.6
			103.0	103.0	98.1	98.0	98.6	98.8
					SS			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			0.172	0.175	0.094	0.090	0.085	0.092
			0.352	0.358	0.140	0.148	0.208	0.202
			0.288	0.280	0.217	0.216	0.119	0.162
					BSS (%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			1.701	1.727	0.925	1.047	0.828	0.895
			1.761	1.793	0.900	0.950	1.047	1.016
			0.932	0.904	0.603	0.646	0.402	0.545
					BH (%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			1.123	1.121	0.571	0.727	3.091	3.001
			-0.209	-0.196	-2.232	-2.286	-0.564	-0.385
			2.996	3.034	-1.933	-2.024	-1.446	-1.170

Table 6. Analysis results of intra-day samples by UPLC-PLS ve UPLC-PCR

					Found (µg/ml)			
Added (µg/ml)			***AA***		***PAR***		***ASP***	
AA	***PAR***	***ASP***	***PLS***	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
10	10	10	10.00	10.01	10.00	10.02	10.16	10.22
20	20	20	20.18	20.18	20.00	20.02	20.13	20.14
30	30	30	30.09	30.09	29.89	29.94	30.19	30.36
					Recovery (%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***		***PLS***	
			100.0	100.1	100.0	100.2	101.6	102.2
			100.9	100.9	100.0	100.1	100.7	100.7
			100.3	100.3	99.6	99.8	100.6	101.2
					SS			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			0.128	0.131	0.093	0.105	0.094	0.110
			0.262	0.268	0.180	0.190	0.255	0.355
			0.539	0.542	0.180	0.193	0.210	0.406
					BSS (%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			1.282	1.308	0.925	1.047	0.925	1.081
			1.298	1.329	0.900	0.950	1.267	1.764
			1.792	1.802	0.603	0.646	0.697	1.336
					BH(%)			
			AA		***PAR***		***ASP***	
			PLS	***PCR***	***PLS***	***PCR***	***PLS***	***PCR***
			0.046	0.081	0.035	0.161	1.602	2.173
			0.900	0.881	-0.020	0.110	0.666	0.702
			0.292	0.300	-0.376	-0.206	0.627	1.210

Tablet analysis

In analyses the real samples, UPLC-PCR, UPLC-PLS and classical-UPLC techniques were applied to the quantitative resolution of PAR, AA and ASP in effervescent tablets. The UPLC chromatograms of tablet samples were illustrated in Figure 2. The experimental results of effervescent tablets were given in Table 7.

The results of all methods were very close to each other as well as to the label value of commercial pharmaceutical dosage form. A good agreement was reported for all the proposed methods.

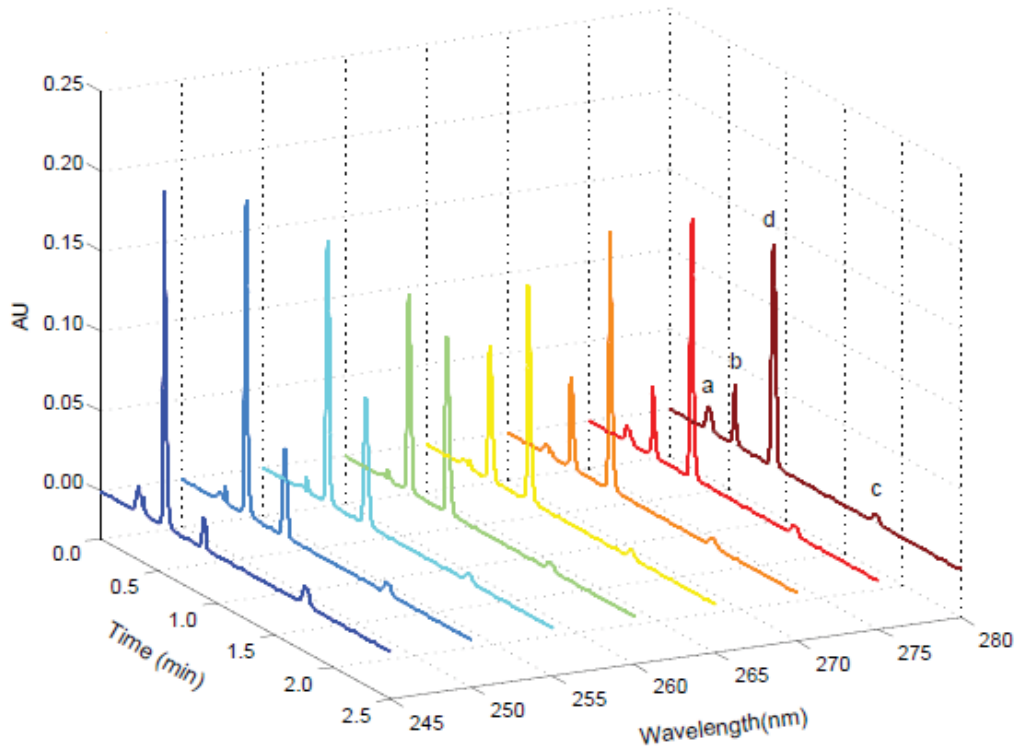

Figure 2. Three dimensional chromatograms of the effervesecent tablet samples at 245, 250, 255, 260, 265, 270, 275 ve 280 nm.

Table 7. Determination results of AA, PAR and ASP in Tablet using UPLC-PLS ve UPLC-PCR

	mg/tablet								
	UPLC-PCR			***UPLC-PLS***			***Classical UPLC***		
	AA	***PAR***	***ASP***	***AA***	***PAR***	***ASP***	***AA***	***PAR***	***ASP***
Mean:	303.4	200.8	303.4	303.2	200.8	302.9	303.5	197.1	304.8
SD	1.78	2.56	1.50	1.65	2.47	1.65	10.47	4.82	8.51
RSD	0.59	1.27	0.50	0.54	1.23	0.54	3.45	2.44	2.79

Conclusions

In this study, UPLC-PCR and UPLC-PLS calibration models were improved for simultaneous quantitative analysis of AA, PAR and ASP in samples. For a comparison of the UPLC-chemometric methods, a classical UPLC method was developed for the analysis of ternary mixtures containing AA, PAR and ASP. A good agreement was observed for the results of the UPLC-chemometric approaches and observed UPLC method. This new application of chemometric calibration techniques to the UPLC data set is an alternative model for the minimization of the experimental errors in chromatographic analysis.

The UPLC-chemometric calibration techniques can be successfully applied to the routine and quality control analysis of AA, PAR and ASP in an effervescent tablet.

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