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DEVELOPMENT AND VALIDATION OF RP-UPLC AND DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR QUANTITATIVE DETERMINATION OF ASCORBIC ACID AND N-ACETYLCYSTEINE IN EFFERVESCENT TABLETS

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ABSTRACT

In this study, reverse phase-ultra performance liquid chromatographic (RP-UPLC) and first derivative spectrophotometric methods were developed and validated for the simultaneous determination of ascorbic acid (AA) and n-acetylcysteine (NAC) quantities in effervescent tablets. Liquid chromatography tests were carried out isocratically with the UPLC BEH C_{18} column. Analyzes were completed in a short time (approximately 6 minutes) with the developed method. UV detector at 220 nm (detection wavelength) was used and the mobile phase was composed of acetonitrile and phosphate buffer (1:99, v/v, pH 2.5). Linearity ranges in methods were 3-60 µg mL^{-1} for AA and 6-84 µg mL^{-1} for NAC. Retention times were 1.59 and 4.61 min for AA and NAC, respectively. First derivative spectrophotometry was employed as a second analytical method and AA and NAC derivative spectra were recorded at 285 nm and 222.5 nm respectively. High recovery values were obtained for AA and NAC. The methods developed were validated for the parameters of linearity, accuracy, precision, specificity, sensitivity, the limit of quantitation, the limit of detection and recovery as defined in the ICH analytical method validation guidelines. Then, the methods were successfully applied to routine analysis for the determination of AA and NAC in commercial effervescent tablets without any interference by the excipients. These methods are also advantageous in that they do not require a pre-separation process for analysis. Furthermore, a large number of samples could be analyzed quickly with this method due to the short chromatographic analysis time. Finally, the results were evaluated statistically and the methods were compared with each other.

KEYWORDS: ***ascorbic acid; n-acetylcysteine; RP-UPLC determination; derivative spectrophotometric determination.***

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INTRODUCTION

AA (Vitamin C) (Figure 1) is involved in many biological processes.[1] it is essential in the human diet and participates in many different physiological and metabolic processes. In the food and pharmaceutical industries, AA content determination is important for quality purposes.[2] NAC (Figure 2) is mainly used as a mucolytic agent in bronchitis or pulmonary diseases. Besides mucolytic effects, it is also an antioxidant and anti-inflammator.[3] in the literature, liquid chromatography and spectroscopic quantitative analysis methods developed for the separate (single) analysis of AA and NAC active ingredients were frequently reported. There are also several quantitative analytical methods with liquid chromatography for these two active ingredients with the combinations of other active ingredients. There are several HPLC quantitative methods developed for the only AA[1,4-6] and for only NAC[3,7] and also several spectroscopic quantitative methods developed for only AA[2, 8-14], HPLC methods for AA [15-22] and NAC [23-25] in combination with other active ingredients, derivative spectroscopy method for only AA[26, 27] and AA[28-35] in combination with other active ingredients. On the other hand, there are no spectroscopic or derivative spectroscopic quantity determination works that have been reported for NAC only. Furthermore, in the literature, there is no liquid chromatographic or derivative spectroscopic quantity analysis method for the simultaneous determination of AA and NAC in pharmaceutical preparations. These studies are first in the literature worked together with RP-UPLC and first derivative spectrophotometry for the simultaneous determination of AA and NAC in the same pharmaceutical product. The aim of this study are to develop simple, fast, and efficient UPLC and first derivative spectrophotometric methods to be applied in the simultaneous determination of AA and NAC in pharmaceutical preparations containing various excipients, without prior sample preparation, to validate these methods in the context of current ICH Analytical Method Validation.[36] In addition, the results obtained from RP-UPLC and derivative spectrophotometric methods were compared with the help of various statistical methods.

EXPERIMENTAL

Instrument

Waters brand Acquity Ultra performance liquid chromatography system was used for chromatographic studies. UPLC system consists of Acquity UPLC sample organizer, Column manager and heater/cooler, Binary Solvent Manager, sample manager, and a UV detector. UPLC was connected to a Samsung Computer and Empower Software was employed. Deionized water obtained from Millipore brand ultra-pure water device was used for all solution preparation processes. Agilent UV-8453 model UV Spectrophotometer device was used for spectrophotometric tests. The Spectrophotometer was connected to a Samsung computer and Vision software and HP Printer was used. 1.0 cm quartz cells were used for the measurements.

Materials and Reagents

AA and NAC certified reference standards were obtained from Sigma-Aldrich Corporation. Mucovit C Effervescent Tablets containing 200 mg AA and 600 mg NAC and produced by Neutec medical company obtained from the local market. Phosphoric acid and acetonitrile are HPLC grade (from Merck) and were used for the preparation of the mobile phase. Analytical grade $NaH_2PO_4.2H_2O$ was used for the buffer solution.

Optimization of Liquid Chromatographic Conditions

Chromatographic studies were carried out using isocratic elution method with UPLC BEH C_{18} column (2.1 x 100 mm) inverse phase column at 30°C. The mobile phase mixture used for separation was tested at different ratios (1:99, 2:98, 5:95, 10:90, etc.). Since separation was realized in a short period time with UPLC, an increase in the organic solution rate caused an interference of the peaks. Therefore, phosphate mixture buffer (pH 2.5) with a 1:99 (v/v) ratio was chosen as the most suitable mobile phase. The mobile phase was prepared on a daily basis and degassed by filtering with a 0.45 µm membrane filter. The flow rate was 0.2 mL/min, injection volume was 2 µL and the analysis period was 6 minutes. While retention time was determined as 1.59 min for AA it was 4.61 min for NAC (Figures 3 and 4). As can be seen from the figure, picks for AA and NAC, which were mixed in effervescent tablets, separated definitely (Figure 5). The method development process was carried out using a UV detector at 220 nm.

System Suitability Parameters

System suitability parameters for chromatographic

method efficiency were calculated in compliance with the recommendations of the Centre for Drug Evaluation and Research (CDER).[37] and the following values were obtained: the selectivity coefficient α: 6.8, the number of theoretical plates, N_{AA}: 4217 and N_{NAC}: 13522, the resolution R_s: 18.4, the symmetry factors, $A_{s(AA)}$: 1.6 and $A_{s(NAC)}$: 1.2, the capacity factors, k_{AA}': 0.48 and k_{NAC}': 3.3, the plate heights, H_{AA}: $1.19.10^{-3}$ cm and H_{NAC}: $3.70.10^{-4}$ cm.

Optimization of Derivative Spectroscopic Conditions

Standard solutions for AA and NAC were prepared in deionized water media for the UV derivative spectrophotometric method developed for comparison purposes. After spectroscopic scanning of these standards within 190-400 nm wavelengths, UV visible region optimum absorption spectra was 280 nm for AA and 200 nm for NAC within spectral bandwidth of 2 nm (Figures 6 and 7). The spectra of these solutions were not changed, indicating that the excipients do not interfere. Maximum wavelengths observed in absorption spectra for both active ingredients do not intervene in the mixture form (Figure 8). On the other hand, first and second derivative spectra were taken to improve resolution results for AA and NAC spectra. First derivative spectra were preferred for all analyses (Figures 11, 12 and 13) since signals decreased and signal/noise ratio got worse in the second derivative spectra (Figures 9 and 10). It was determined that AA and NAC absorbed in close λ_{max} values and both have broad absorption bands. Therefore, the first derivative spectrometry method was developed instead of UV spectrophotometry. Working wavelengths were chosen as 285 nm for AA and 222.5 nm for NAC as minimum wavelengths. In this way, overlapped bands were separated, sharper peaks were obtained and possible impurities were avoided. Calibration curves used in derivative spectrometry were drawn for the absorbance values obtained from the first derivative signals for AA and NAC concentrations. $dA/d\lambda$ values measured from calibration curves obtained with the first derivative spectrometry method were found with a pick to zero technique at minimum wavelengths at 285 nm and 222.5 nm for AA and NAC respectively.

Preparation of Stock and Standard Solutions

200 µg mL^{-1}'lık AA and 600 µg mL^{-1}'lık NAC stock solutions for LC and derivative UV analysis were prepared using deionized water. Stock solutions were kept at the refrigerator at 4°C for a period of one week without any deformation and they were taken out before the injection process. In the dilution process, standard solutions at 7 different concentration levels were prepared within 3-60 µg mL^{-1} concentration ranges for AA and within 6-84µg mL^{-1} ranges for NAC. During our studies, working standard solutions were prepared daily. After that these standard solutions were measured separately and calibration curves were formed for LC and derivative UV studies. For intra-day and inter-day precision measurements, solutions at 12, 36, 60 µg mL^{-1} concentrations for AA and at 12, 36, 84 µg mL^{-1} concentrations for NAC were chosen as working standards. These standard solutions were also kept at the refrigerator before the analysis. Recovery standard solutions were prepared at 10 different concentration levels from the stock solutions for both chromatographic and derivative spectroscopic analysis by diluting with deionized water. While the concentration range for AA is 3-60 µg mL^{-1} (3, 12, 18, 24, 36, 40, 42, 50, 54 and 60 µg mL^{-1}), it was 6-84 µg mL^{-1} for NAC (6, 12, 15, 18, 36, 39, 42, 48, 60 and 84 µg mL^{-1}).

Preparation of Pharmaceutical Samples

Both of the quantitative analysis methods developed applied to Mucovit C Effervescent trade tablet preparation, containing AA and NAC. To prepare stock sample solutions to be used in LC and derivative UV analysis, effervescent tablets containing AA (200 mg/tab) and NAC (600 mg/tab) active ingredients were powdered and diluted to obtain 200 µg mL^{-1} AA and 600 µg mL^{-1} NAC concentrations. After that, these stock sample solutions diluted at 1/10 ratios, and 6 pharmaceutical sample solutions at 20 µg mL^{-1} AA and 60 µg mL^{-1} NAC concentrations were prepared daily.

Preparation of Standards with Standard Addition Method

New standard solutions were prepared for standard addition method by diluting from the same stock solutions. 5 different standard solutions were prepared for AA by taking 16, 12, 10, 8 and 2 µg mL^{-1} from the stocks and 4, 8, 10, 12 and 18 µg mL^{-1} from the trade tablets respectively and they are completed to the desired quantities by adding water. 5 different standard solutions were prepared for NAC by taking 48, 36, 30, 24 and 6 µg mL^{-1} from the stocks and 12, 24, 30, 36 and 54 µg mL^{-1} from the trade tablets respectively and they are completed to the desired quantities by adding water. In this way, each standard solution

contained 20 µg mL^{-1} AA and 60 µg mL^{-1} NAC in total. These standards were kept in the refrigerator at 4 °C and brought to room temperature before the analysis. Before giving in to the UPLC system, all the solutions were filtered with a 0.45-micron membrane filter. Spectroscopic measurements for the same solutions were made with 10 mm quartz cells.

RESULTS AND DISCUSSION

Analytical Method Validation

Analytical method validation for AA and NAC were made in compliance with ICH parameters. Linearity, accuracy, precision, recovery, LOQ (the Limit of Quantitation), LOD (the Limit of Detection) and selectivity parameters were calculated for validation purposes.

Linearity

Calibration curves were drawn for standard solutions containing 3, 12, 24, 36, 42, 54 and 60 µg mL^{-1} AA and 6, 12, 18, 36, 42, 60 and 84 µg mL^{-1} NAC (n= 6). After that, regression equations were defined from the data obtained using the least square regression method. Linearity data were computed on a personal computer on Microsoft Excel (Version 2003, Microsoft Co., and Redmond, USA). Statistical results, regression equations, correlation coefficients, RSD (Relative Standard Deviation) values, linearity intervals, S_b and S_m values obtained from both UPLC and derivative spectroscopy methods were summarized in Tables 1 and 2. Furthermore, LOQ and LOD parameters showing the sensitivity of AA and NAC measurements are given in the same tables. According to the results given in the tables, good linearity values were obtained for both active ingredients.

Precision

Three different concentration levels used for calibration curves formed for both methods were chosen (12, 36, 60 µg mL^{-1} for AA and 12, 36, 84 µg mL^{-1} for NAC) and six measurements were made for intra-day and inter-day precision measurements. All RSD % values were calculated for precision parameter and it was seen that these values are far above 10 % for both AA and NAC (Tables 3 and 4). Maximum calculated RSD % values are less than 3.77 and this indicates that the methods have high precision (repeatability) values.

Accuracy

For recovery measurements, standard solutions at 10 different concentrations were prepared from the same stock solutions and very good recovery (about 94% and above) and precision results (the relative error is about ±3% and below) were obtained. Results obtained from both analysis methods were summarized in Tables 5 and 6. As can be seen from the tables all the recovery values are about 100%. Recovery results obtained for both active ingredients from UPLC and UV analysis methods were given in Tables 7 and 8 (about 97% and above). We should also have to determine if the excipients contained in the preparation affect both analytical methods. For this purpose, recovery results obtained from trade preparations containing AA and NAC (AA: 20 µg mL^{-1} and NAC: 60 µg mL^{-1}) from both methods are given in Tables 9 and 10. Very high recovery values were obtained from both analysis methods and accuracy (relative error) data was ≤ 3.43. All these results indicate that excipients in the preparation have no significant effect on the results.

Selectivity

Ingredients in the mixture format were injected into the system and separate peaks for each ingredient were defined in the UPLC method developed for the simultaneous analysis of both active ingredients. Active ingredients in the chromatograms obtained from standards and trade tablet solutions appeared in the same retention times and therefore the method can be accepted as specific. Furthermore, no interference peak stem from excipients have shown in the chromatograms obtained from the tablets, in other words, a good separation was obtained for the excipients.

Robustness

To check the robustness of the UPLC method developed, the effect of the minor changes in the method parameters was examined. When the results obtained after the changes made on the ACN ratio added for mobile phase, buffer pH, buffer concentration and flow rate were compared with the results at the optimum conditions, there is no difference between them and this indicated that the method robust.

Stability

It was found that AA and NAC water solutions are stable at room temperature, under sunlight and at night for a period of 24 hours and also, they are not subject to a meaningful deformation when they are kept at +4 °C for a period of 2 months.

STATISTICAL ANALYSIS

To compare UPLC and first derivative spectrophotometric quantitative determination methods, statistical one-way analysis of variance (ANOVA) was applied to the obtained results for commercial tablet formulations using the SPSS16 program. The results were shown in Tables 11 and 12 and within a 95 % confidence interval (P= 0.05) there is no significant statistical difference between UPLC and first derivative spectrophotometric quantitative determination methods for AA. But there is a significant difference between the two quantitative determination methods used for NAC.

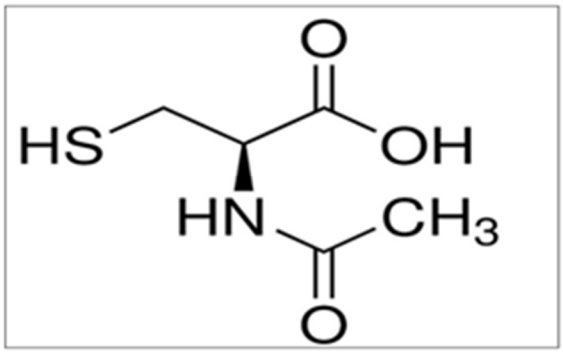

Figure 1
The structural formula of AA

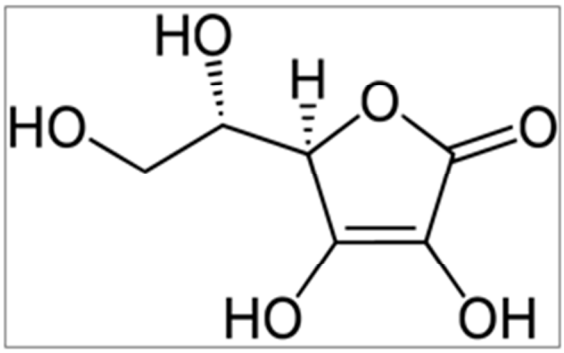

Figure 2
The structural formula of NAC

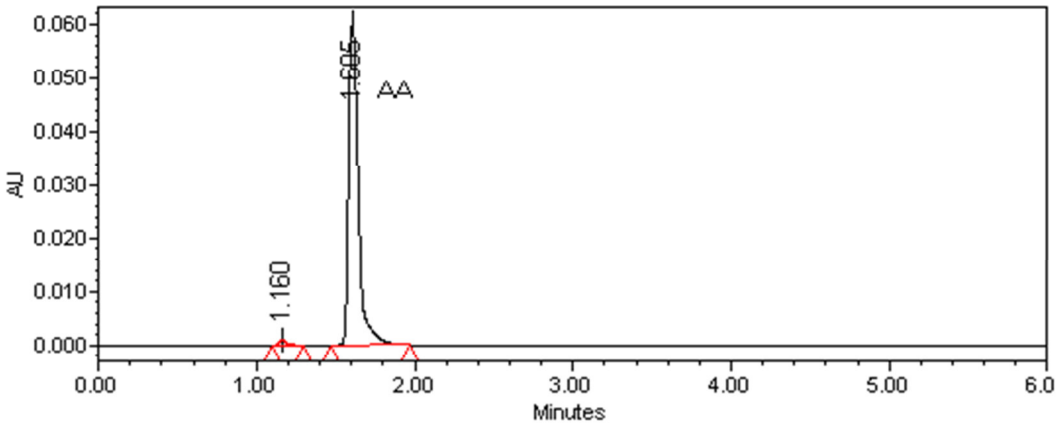

Figure 3
AA chromatogram of 20 µg ml $^{-1}$

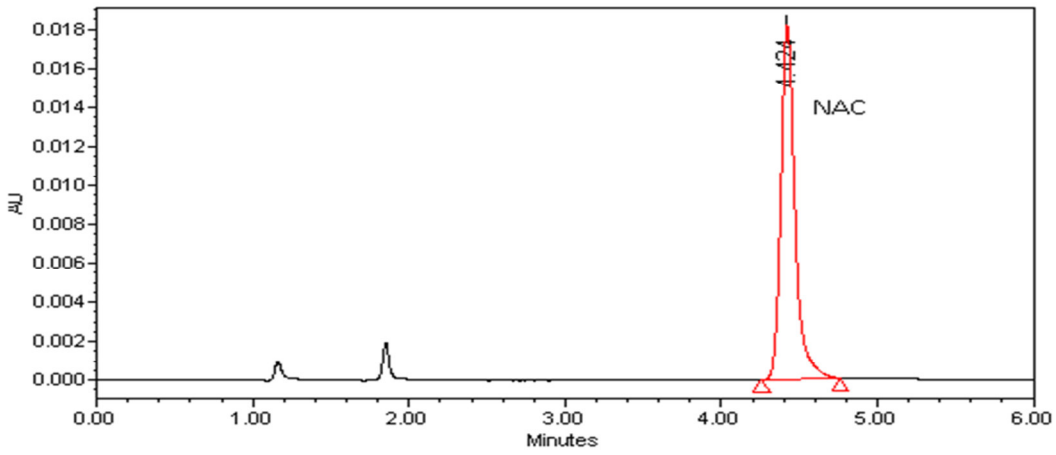

Figure 4
NAC chromatogram of 60 µg ml $^{-1}$

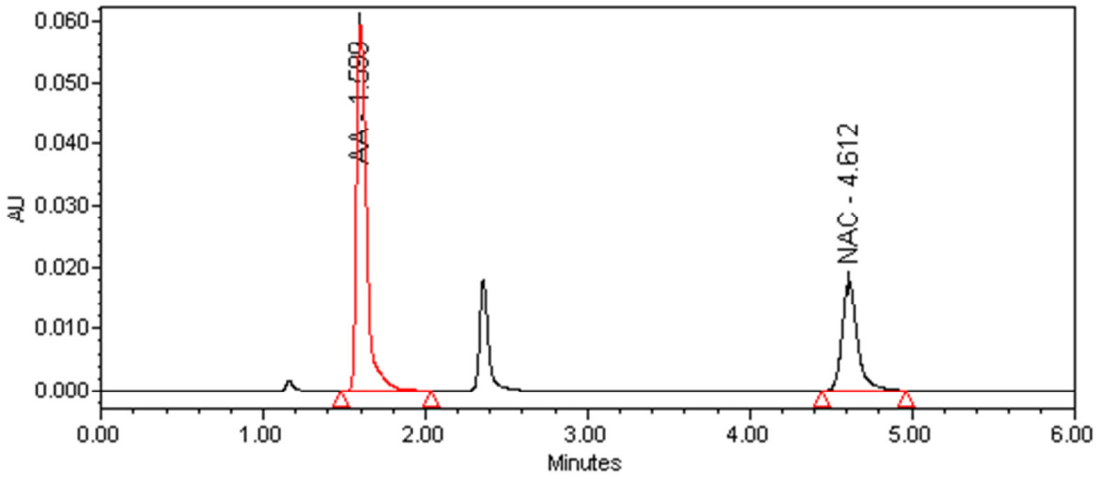

Figure 5
Commercial effervescent tablet chromatogram of 20 µg ml $^{-1}$ AA and 60 µg ml $^{-1}$ NAC

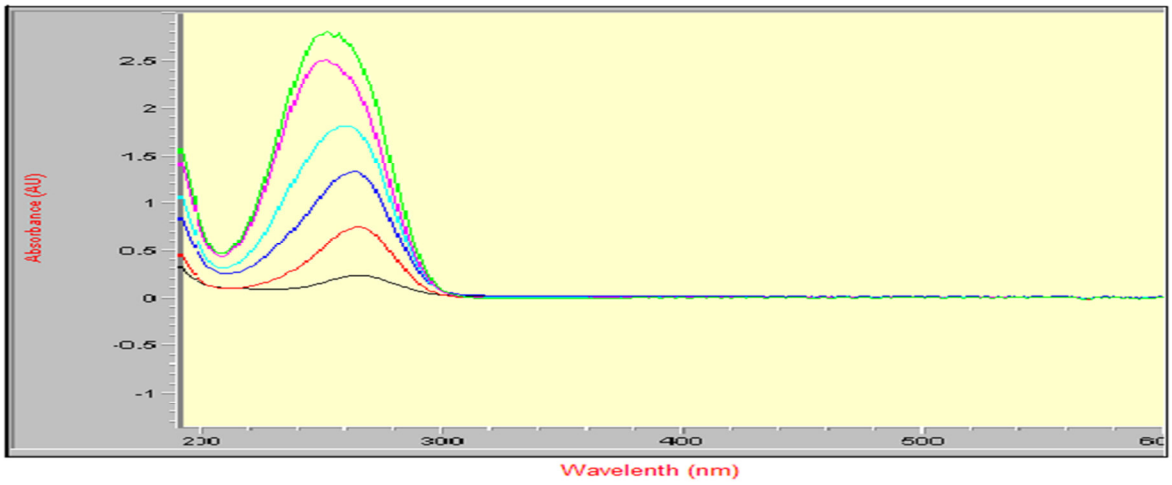

Figure 6
UV absorbance spectra of AA

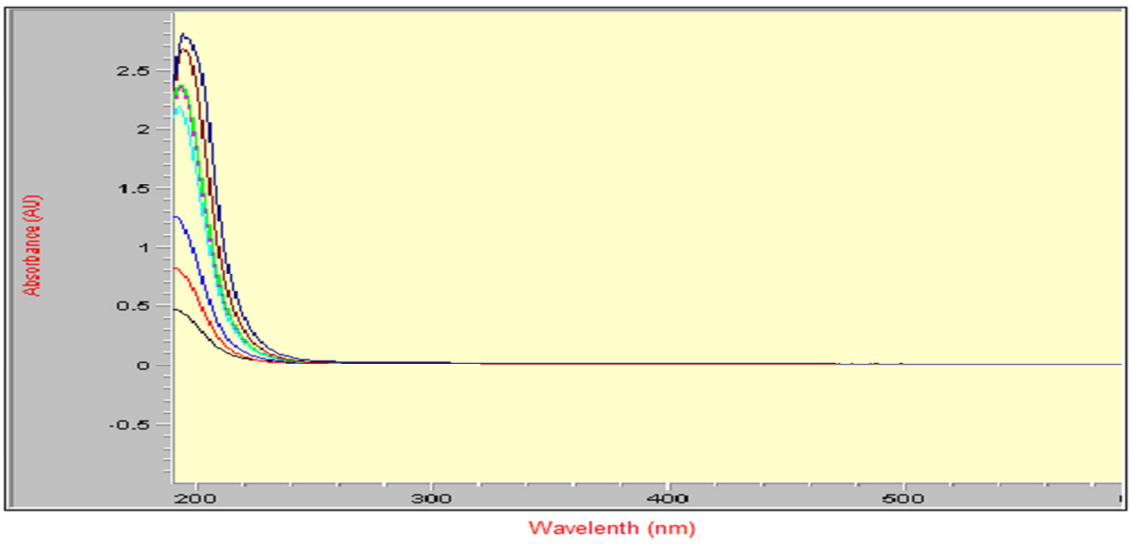

Figure 7
UV absorbance spectra of NAC

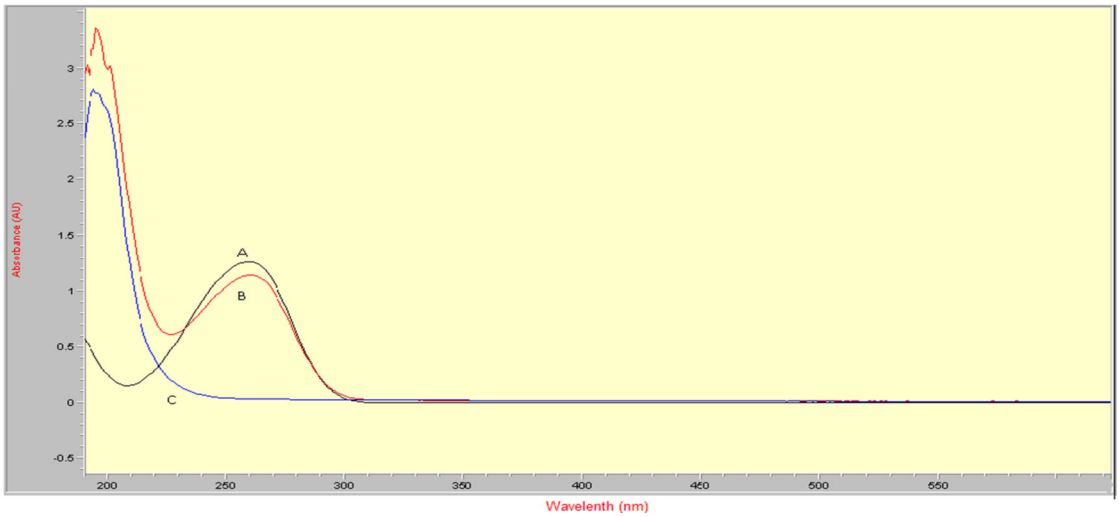

Figure 8
UV absorbance spectra of AA (A:—280 nm), NAC (C:—200 nm) and commercial effervescent tablet (B:—mixture)

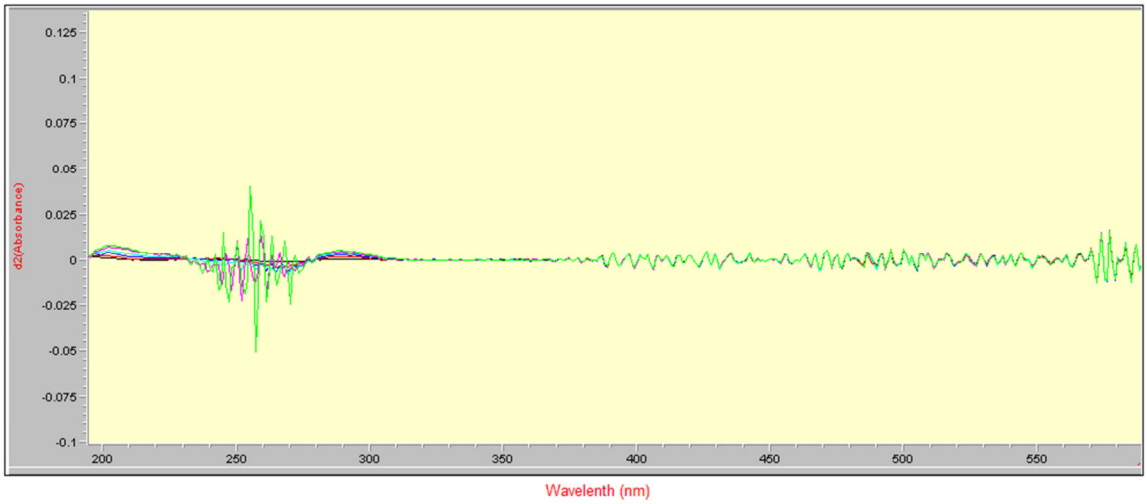

Figure 9
Second-order derivative spectra of AA

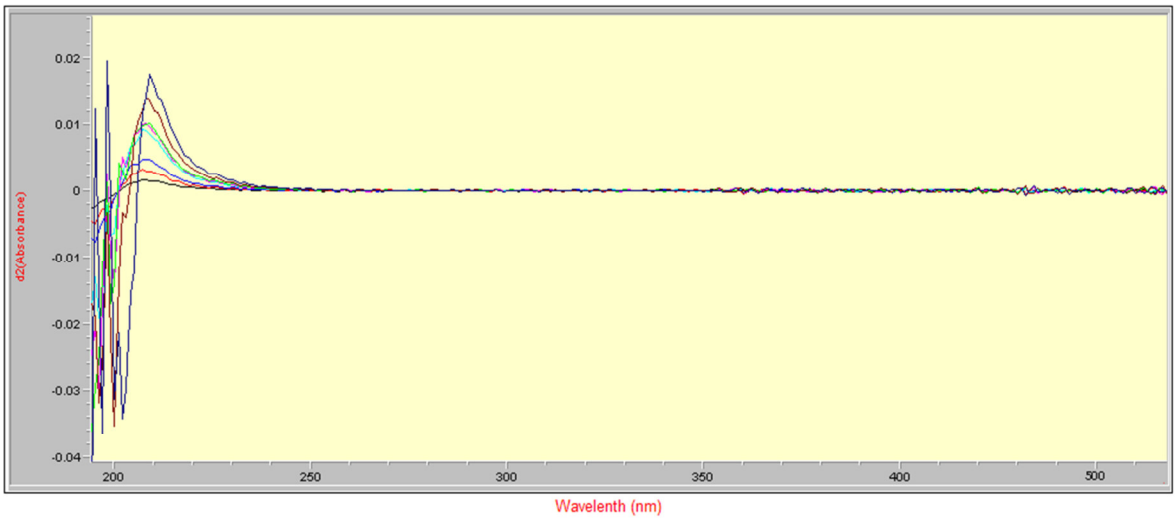

Figure 10
Second-order derivative spectra of NAC

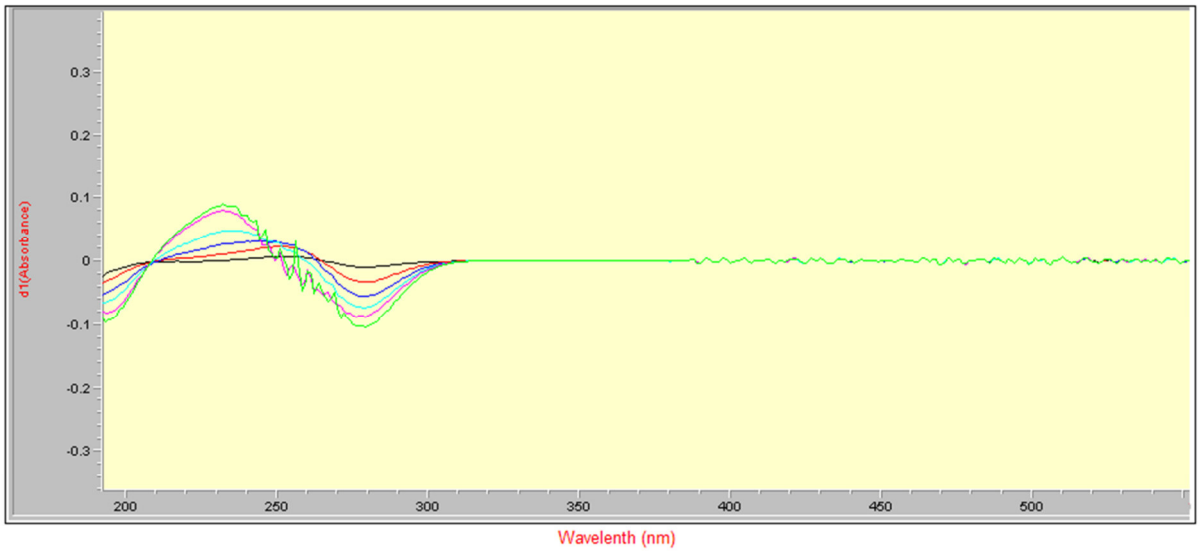

Figure 11
First-order derivative spectra of AA

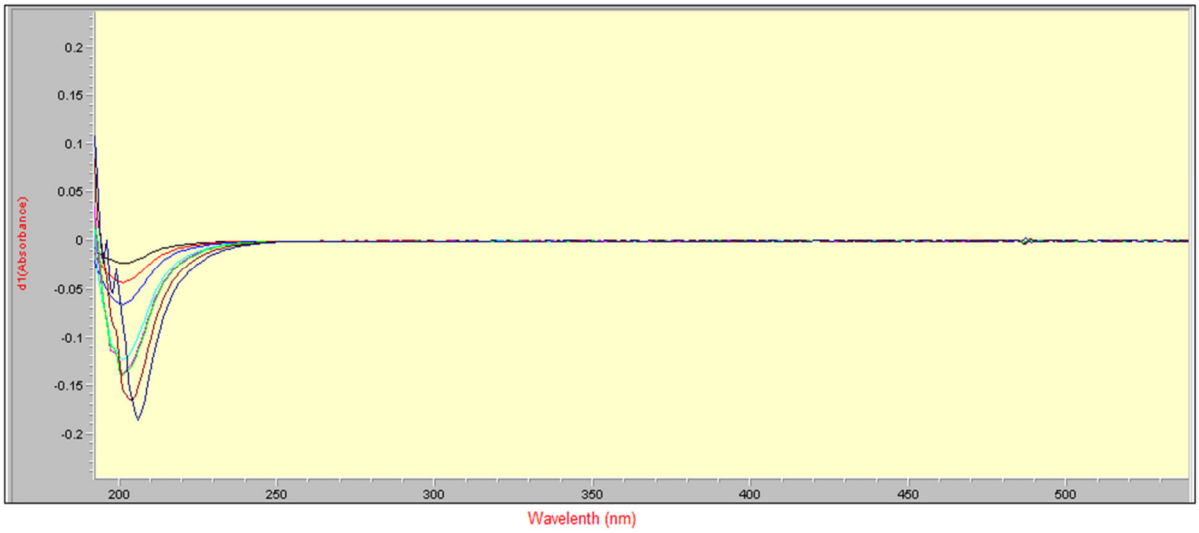

Figure 12
First-order derivative spectra of NAC

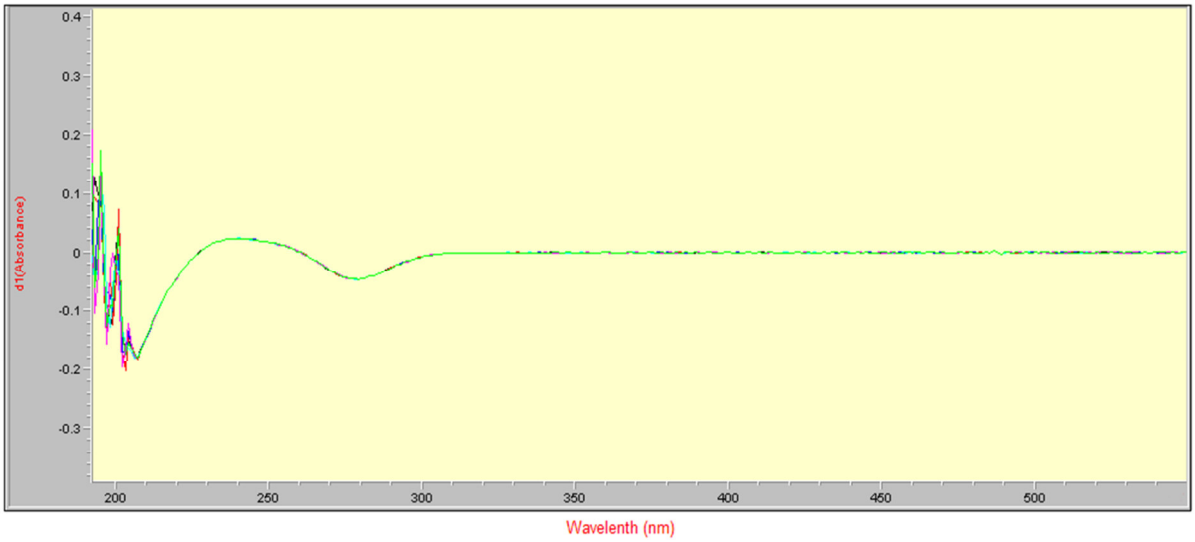

Figure 13
First-order derivative spectra of commercial effervescent tablet

Table 1
Features of the calibration curves of AA and NAC for UPLC determination (n= 6)

	In the mobile phase solvent medium	
	AA	**NAC**
Regression equation	y= 12868x-4939.20	y= 1880.4x-612.02
The correlation coefficient (r)	0.9998	0.9998
RSD* (%)	0.15-0.90	0.22-1.07
Linear range (µg mL^{-1})	3-60	6-84
S_b	1851.73	358.45
S_m	48.26	7.96
LOQ (µg mL^{-1})	2.99	4.50
LOD (µg mL^{-1})	0.90	1.35

Relative standard deviation, RSD = Standard deviation/Mean value x 100, The standard deviation of the intercept of the regression line (S_b) and the standard deviation of a slope, (S_m). The limit of detection, LOD = 3.Standard deviation/slope and the limit of quantification, LOQ = 10.Standard deviation/slope.

Table 2
Features of the calibration curves of AA and NAC for first derivative Spectrophotometric determination (n=6)

	First-order derivative	
	AA	**NAC**
Regression equation	y= -0.00124x-0.0180	y= -0.000356x-0.00132
The correlation coefficient (r)	0.9820	0.9990
RSD* (%)	0.26-3.03	0.18-1.07
Linear range (µg mL^{-1})	3-60	6-84
S_b	1.64	0.2
S_m	0.00049	0.0000596
LOQ (µg mL^{-1})	0.62	0.32
LOD (µg mL^{-1})	0.19	1.06

Table 3
The precision of AA and NAC for UPLC method (n=6)

Standard solutions of AA	**Intra-day**			**Inter-day**		
Concentration (µg mL^{-1})	**X**	**SD**	**RSD**	**X**	**SD**	**RSD**
12	145652	214.29	0.15	143846	3985.60	2.77
36	458806	900.29	0.20	460907	3217.79	0.70
60	764174	2948.28	0.39	770546	4274.41	0.55
Standard solutions of NAC	**Intra-day**			**Inter-day**		
Concentration (µg mL^{-1})	**X**	**SD**	**RSD**	**X**	**SD**	**RSD**
12	21876	176.61	0.81	21552	399.19	1.85
36	67587	350.79	0.52	67439	555.16	0.82
84	156440	521.48	0.33	156697	796.42	0.51

X: mean value, SD: standard deviation, RSD: relative standard deviation (100.SD/ mean).

Table 4

The precision of AA and NAC for the derivative spectrophotometric method (n=6).

Standard solutions of AA	Intra-day			Inter-day		
Concentration (μg mL^{-1})	X	SD	RSD	X	SD	RSD
12	-0.03653	0.00016	-0.43	0.03829	0.00144	-3.77
36	-0.06564	0.00023	-0.36	0.06659	0.00063	-0.94
60	-0.08982	0.00044	-0.49	0.08986	0.00072	-0.80
Standard solutions of NAC	**Intra-day**			**Inter-day**		
Concentration (μg mL^{-1})	**X**	**SD**	**RSD**	**X**	**SD**	**RSD**
12	0.005290	0.000030	-0.56	0.005373	0.00010	-1.83
36	0.014573	0.000032	-0.22	0.015163	0.00050	-3.28
84	0.030849	0.000143	-0.46	0.031942	0.00111	-3.47

Table 5

The data of recovery for AA and NAC standard for UPLC determination

AA				NAC			
Added (μg mL^{-1})	Founded (μg mL^{-1})	Recovery* (%)	Accuracy** (Relative error, %)	Added (μg mL^{-1})	Founded (μg mL^{-1})	Recovery* (%)	Accuracy** (Relative error, %)
3	3.09	103.00	3.00	6	5.88	98.00	-2.00
12	12.23	101.92	1.92	12	11.63	96.92	-3.08
18	18.16	100.89	0.89	15	14.6	97.33	-2.67
24	23.74	98.92	-1.08	18	17.59	97.72	-2.28
36	35.44	94.44	-1.56	36	35.15	97.64	-2.36
40	40.31	100.78	0.78	39	38.67	99.15	-0.85
42	42.28	100.67	0.67	42	41.66	99.19	-0.81
50	49.83	99.66	-0.34	48	47.57	99.10	-0.90
54	54.44	100.81	0.81	60	60.65	101.08	1.08
60	59.86	99.77	-0.23	84	83.9	99.88	-0.12

Recovery = Found concentration / Added concentration x 100, **Accuracy (relative error) = [(found-added)/added] x 100

Table 6

The data of recovery for AA and NAC standard for first-order determination

AA				NAC			
Added (μg mL^{-1})	Founded (μg mL^{-1})	Recovery* (%)	Accuracy** (Relative error, %)	Added (μg mL^{-1})	Founded (μg mL^{-1})	Recovery* (%)	Accuracy** (Relative error, %)
3	3.03	100.97	0.97	6	5.97	99.42	-0.58
12	12.18	101.52	1.52	12	12.02	100.19	0.19
18	18.33	101.82	1.82	15	15.16	101.06	1.06
24	24.62	102.60	2.60	18	18.44	102.47	2.47
36	37.03	102.87	2.87	36	35.97	99.93	-0.07
40	41.18	102.96	2.96	39	39.62	101.58	1.58
42	42.96	102.29	2.29	42	42.04	100.10	0.10

50	50.82	101.65	1.65	48	48.46	100.95	0.95
54	52.82	97.81	-2.19	60	59.17	98.62	-1.38
60	58.43	97.38	-2.62	84	82.13	97.77	-2.23

Table 7

The data from the standard addition method for AA and NAC for UPLC determination (n=6)

Added tablet ($\mu g\ mL^{-1}$)	Added AA standard ($\mu g\ mL^{-1}$)	Founded ($\mu g\ mL^{-1}$)	Founded ($\mu g\ mL^{-1}$)	Added tablet ($\mu g\ mL^{-1}$)	Added NAC standard ($\mu g\ mL^{-1}$)
12	48	19.20	59.25	4	16
24	36	19.61	60.37	8	12
30	30	19.38	60.53	10	10
36	24	18.88	60.92	12	8
54	6	19.94	62.20	18	2
Found mean concentration (X, $\mu g\ mL^{-1}$)		19.40	60.65		
Standard deviation (SD, $\mu g\ mL^{-1}$)		0.40	1.06		
Relative standard deviation (RSD, %)		2.08	1.75		
Recovery (%)		97.01	101.09		

Table 8

The data from the standard addition method for AA and NAC for derivative order spectrophotometric determination (n=6)

Added tablet ($\mu g\ mL^{-1}$)	Added AA standard ($\mu g\ mL^{-1}$)	Founded ($\mu g\ mL^{-1}$)	Founded ($\mu g\ mL^{-1}$)	Added tablet ($\mu g\ mL^{-1}$)	Added NAC standard ($\mu g\ mL^{-1}$)
12	48	19.34	57.07	4	16
24	36	19.45	60.44	8	12
30	30	20.51	59.98	10	10
36	24	20.55	58.07	12	8
54	6	19.66	58.55	18	2
Found mean concentration (X, $\mu g\ mL^{-1}$)		19.90	58.82		
Standard deviation (SD, $\mu g\ mL^{-1}$)		0.59	1.38		
Relative standard deviation (RSD, %)		2.94	2.35		
Recovery (%)		99.51	98.04		

Table 9

The recovery from commercial tablet solution for AA and NAC for UPLC method (n=6)

AA				NAC			
Added (µg mL^{-1})	**Founded (µg mL^{-1})**	**Recovery (%)**	**Accuracy (Relative error, %)**	**Added (µg mL^{-1})**	**Founded (µg mL^{-1})**	**Recovery (%)**	**Accuracy (Relative error, %)**
20	20.17	100.87	0.87	60	59.97	99.95	-0.05
20	20.11	100.57	0.57	60	59.15	98.58	-1.41
20	20.32	101.60	1.60	60	59.87	99.78	-0.22
20	20.08	100.39	0.39	60	59.17	98.62	-1.38
20	20.60	102.98	2.98	60	61.27	100.45	0.45
20	20.56	102.81	2.81	60	60.23	100.39	0.39
X		101.54				99.63	
SD		1.13				0.83	
RSD		1.12				0.84	

Table 10

The recovery from commercial tablet solution for AA and NAC for the derivative spectrophotometric method (n=6)

AA				NAC			
Added (µg mL^{-1})	**Founded (µg mL^{-1})**	**Recovery (%)**	**Accuracy (Relative error, %)**	**Added (µg mL^{-1})**	**Founded (µg mL^{-1})**	**Recovery (%)**	**Accuracy (Relative error, %)**
20	20.51	102.55	2.55	60	61.99	103.31	3.31
20	20.33	101.64	1.64	60	62.06	103.43	3.43
20	19.34	96.69	-3.31	60	62.83	102.47	2.47
20	20.55	102.74	2.74	60	61.48	102.19	2.19
20	20.10	100.52	0.52	60	61.02	101.70	1.70
20	19.34	96.70	-3.30	60	60.81	101.34	1.34
X		100.14				102.41	
SD		2.78				0.84	
RSD		2.78				0.82	

Table 11

Descriptives for statistical one-way ANOVA test

		n	**Mean**	**Std. Deviation**	**Std. Error**	**Minimum**	**Maximum**
AA	UPLC	6	101.54	1.13	0.46	100.39	102.98
	UV	6	100.14	2.78	1.14	96.69	102.74
	Total	12	100.84	2.15	0.62	96.69	102.98
NAC	UPLC	6	99.63	0.84	0.34	98.58	100.45
	UV	6	102.41	0.84	0.34	101.34	103.43
	Total	12	101.02	1.66	0.48	98.58	103.43

Table 12
Statistical one way ANOVA test data obtained from tablet preparations (P= 0.05 and 95% Confidence level) ($F_{tabulated}$ = 4.96)

		Sum of Squares	df	Mean Square	F	Sig.
AA	**Between Groups**	5.85	1	5.85	1.30	0.28
	Within Groups	45.10	10	4.51		
	Total	50.95	11			
NAC	**Between Groups**	23.16	1	23.16	32.85	0.00
	Within Groups	7.05	10	0.71		
	Total	30.20	11			

CONCLUSION

Quantitative analysis methods such as UPLC and derivative spectrometric methods which are validated, easy to apply and produce correct results in a short period of time, are frequently employed in quality control laboratories. In this study, UPLC and derivative-UV methods were developed for the simultaneous determination of AA and NAC active ingredients within the trade tablet preparations containing both of them. Thanks to the method developed in UPLC with isocratic elution, the analysis was completed in a very short period time (about 6 minutes). Since little time ~~is~~ was required for analysis, a lot of samples could be analyzed in a short period time. On the other hand, the derivative spectrometry method developed for comparison purposes has the advantage of not requiring a pre-separation process for the mixture analysis. The methods developed successfully were applied to the tablet preparations containing both AA and NAC active ingredients. Results showed that both methods can be applied successfully for the analysis of both pharmaceutical preparations. Methods developed were validated according to the linearity, precision, accuracy, recovery, sensitivity, selectivity, robustness and stability parameters as defined in the ICH analytical method validation guide. Low RSD and high recovery values obtained indicate that both analysis methods have very good reproducibility and therefore good precision, accuracy, and high sensitivity. Furthermore, these methods are advantageous for analysis since they do not require a pre-separation process for mixture analysis and have cost-effective budgets. These methods were developed to be practical, selective, linear and repeatable and they can be applied easily for the routine analysis of AA and NAC active ingredients in trade preparations.

AUTHORS CONTRIBUTION STATEMENT

M. Ozaraz planned and carried out the experiments, collected and analyzed the data about this work and performed the analytic calculations. Dr. A. Kucuk Tunca interpreted the results, performed statistical analyses, drafted the article and contributed final approval of the version to be published.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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