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Original article

SPECTROPHOTOMETRIC AND LIQUID CHROMATOGRAPHIC DETERMINATION OF ACEMETACIN IN PHARMACEUTICAL FORMULATIONS

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Abstract

In present study, three new spectrophotometric methods, original UV spectrophotometry, first and second order derivative UV spectrophotometry and a new liquid chromatographic method were developed for the determination of acemetacin in pharmaceutical preparations. In original UV spectrophotometry, absorbances were measured at 280,0 nm in the zero order UV spectra of the solution of acemetacin in 0,1N NaOH in the range of 210 - 325 nm. In first derivative UV spectrophotometry, dA/dλ values were measured at 240.0 nm in the first derivative UV spectra of the solution of acemetacin in 0,1 N NaOH in the range of 230 - 325 nm (Δλ= 2 nm). In second derivative UV spectrophotometry $d^2A/d\lambda^2$ values were measured at 274.0 nm in the second derivative UV spectra of the solution of acemetacin in 0,1 N NaOH in the range of 240 - 325 nm (Δλ= 2 nm). Linearity range was found as 8.0 – 85.0 μg/mL in original UV spectrophotometric and first order derivative UV spectrophotometric methods and 15.0 – 85.0 μg/mL in second order derivative UV spectrophotometric method. Mean recoveries and the relative standard deviations of the methods were found as 100.64 % and 0.95 % in original UV spectrophotometic method 100.36 % and 0.62 % in first derivative UV spectrophotometric method and, 100.45 % and 1.10 % in second derivative UV spectrophotometric method respectively. Also, a new HPLC method was developed. In this method, ACE C18 analytical column and a mobile phase composed of acetonitrile – water (80:20, v/v) at a flow rate of 1.0 mL/min was used for the optimal chromatographic separation using UV detection at 280 nm. Dienogest was used as internal standard. All the methods developed were successfully applied to two tablet formulations commercially available in Turkish drug market. All the results were compared statistically with each other.

Key words: *Acemetacin, Spectrophotometry, HPLC, Determination, Pharmaceutical preparation*

Asemetasin'in Farmasötik Preparatlarda Spektrofotometrik ve Sıvı Kromatografik Yöntemlerle Miktar Tayini

Bu çalışmada asemetasin'in farmasötik preparatlarda miktar tayini için üç yeni spektrofotometrik yöntem, orijinal UV spektrofotometri, birinci ve ikinci türev spektrofotometri, geliştirilmiştir. Orijinal UV spektrofotometride absorbans değerleri, asemetasin'in 0,1 N NaOH içerisindeki çözeltilerinin 210-325 nm aralığındaki UV spektrumlarında 280.0 nm de ölçülmüştür. Birinci türev UV spektrofotometride, dA/dλ değerleri, asemetasin in 0,1 N NaOH içerisindeki çözeltilerinin 230-325 nm aralığındaki birinci türev UV spektrumlarında (Δλ= 2 nm) 240.0 nm de ölçülmüştür. İkinci türev UV spektrofotometride $d^2A/d\lambda^2$ değerleri asemetasin'in 0,1 N NaOH içerisindeki çözeltilerinin 240-325 nm aralığındaki ikinci türev UV spektrumlarında (Δλ= 2 nm) 274.0 nm de ölçülmüştür. Çalışmada doğrusal çalışma aralığı orijinal UV spektrofotometri ve birinci türev spektrofotometri için 8.0 – 85.0 μg/mL, ikinci türev spektrofotometri için ise 15.0 – 85.0 μg/mL olarak bulunmuştur. Yöntemlerdeki ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla orijinal UV spektrofotometrik yöntemde % 100.64 ve % 0.95, birinci türev UV spektrofotometrik yöntemde % 100.36 ve % 0.62 ve, ikinci türev UV spektrofotometrik yöntemde % 100.45 ve % 1.10 olarak bulunmuştur. Ayrıca tarafımızdan yeni bir YPSK yöntemi *geliştirilmiştir. Bu yöntemde, optimal kromatografik ayırım, ACE C18 kolonu ve 1mL/dk akış hızında asetonitril-su (80:20, v/v) mobil fazı ve 280 nm'de deteksiyonla sağlanmıştır. İç standart olarak dienogest kullanılmıştır. Geliştirilen yöntemler Türkiye ilaç piyasasında bulunan iki adet tablet formulasyonuna başarıyla uygulanmıştır. Elde edilen tüm sonuçlar kendi aralarında istatistiksel olarak karşılaştırılmıştır.*

Anahtar kelimeler: *Asemetasin , Spektrofotometri, YPSK, Miktar tayini, Farmasötik preparat*

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INTRODUCTION

Acemetacin (Figure 1) is a non-steroidal anti-inflammatory drug, used for the treatment of osteoarthritis, rheumatoid arthritis, lower back pain, and relieving post-operative pain. Acemetacin, a glycolic acid ester of indometacin, acts as a prodrug; in the body, it is metabolized to indometacin, which then acts as an inhibitor of cyclooxygenase, producing the anti-inflammatory effects.

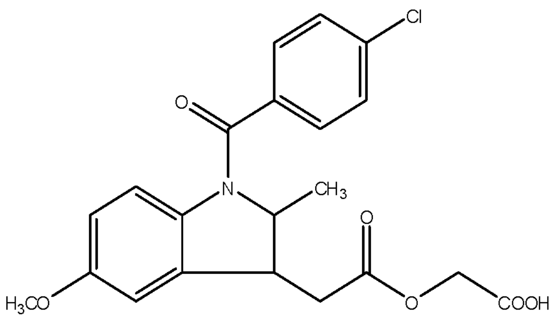

Figure 1. Acemetacin

In previous studies; the determination of acemetacin in pharmaceutical preparations containing only acemetacin was realized by using several methods including spectrophotometry (1,2), micellar liquid chromatograpy (3,4) and FIA (5). The determination of acemetacinin in biological liquids was made by using HPLC (6-13) and voltammetry (14,15). Simultaneous determination of acemetacin and indomethacin in tablets was realized by using second derivative spectrophotometry (1) and a chemometric technique (2). However, no information concerning with the determination of acemetacin in pharmaceutical preparations by using original UV spectrophotometry and derivative UV spectrophotometric methods could be seen in the literatures.

EXPERIMENTAL

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

An Agilent Technologies HP 1100 chromatographic system was used equipped with a model series of G13 79A degasser, G1311A quaternary pump, 61313A injector and G1315B DAD detector. ACE C18 column 250 x 4.6 mm, 5 μm particle sized was used.

Materials

Acemetacin was kindly donated by BAYER Pharm.Ind., Turkey and used without further purification.

All the materials used in the spectrophotometric analysis were of analytical reagent grade. Acetonitrile, used in HPLC method, was of chromatographic reagent grade.

Standard solutions

125 mg / 500 mL solutions of acemetacin were prepared in 0.1 N NaOH for spectrophotometric methods. For HPLC, 25 mg / 100 mL solution of acemetacin and 5 mg / 100 mL solution of dienogest (IS) were prepared in acetonitrile separately.

Commercial pharmaceutical preparations assayed

Name	*Content*	*Batch no.*	*Firm*
RANTUDİL Fort	60 mg acemetacin /capsul	*76390A*	Bayer
RANTUDİL Retard	90 mg acemetacin /capsul	*6R239*	Bayer

Sample preparation

a) For spectrophotometric procedures: The content of 20 tablets were accurately weighed and powdered in a mortar. An amount of mass equivalent to one tablet was weighed in 50 mL volumetric flask and diluted to volume with 0,1 N NaOH After 45 min of mechanically shaking, solution was filtered through 4.5 μm milipore filter. Portion of the initial 5 mL was discarded and 1 ml of filtered solution was put into a 25 ml volumetric flask and the volume was completed to 25 mL with the same solvent. Final solution was used for the determination.

b) For HPLC procedure: The content of 20 tablets were accurately weighed and powdered in a mortar. An amount of mass equivalent to one tablet 50 mL volumetric flask and diluted to volume with acetonitile After 15 min of mechanically shaking and keeping 1 min. ultrasonic bath, solution was filtered through 4.5 μm milipore filter. Portion of the initial 5 mL was discarded and 1 ml of filtered solution was put into a 25 ml volumetric flask and 2.5 mL of standard solution of internal standard (dienogest) was added and, then the volume was completed to 25 mL with the same solvent. Final solution was used for the determination.

RESULTS

Original UV spectrophotometry

There are two maxima (225.0 and 280.0 nm) in zero-order UV spectra of the solution of acemetacin (**ACE**) in 0,1 N NaOH in the range of 210-325 nm (Figure 2). The determination of **ACE** can be realized by measuring the absorbances at 280 nm and using the calibration curve prepared by plotting the absorbances versus ten different concentrations of standard substance. Linearity range according to the Beer's law was found as 8.0 – 85.0 μg/mL in the method. LOQ was 8.0 μg/mL and LOD was calculated as 2.66 μg/mL by using the following equation; 3.3 SD/m (SD=Standard deviation, m=slope). Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 2.

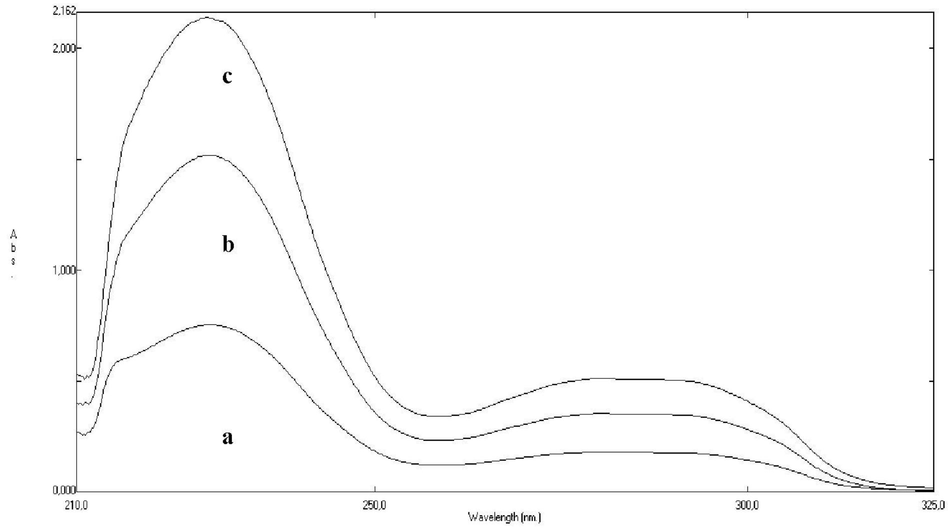

Figure 2. UV spectrum of the a)10 μg/mL b) 20 μg/mL c)30 μg/mL solution of **ACE** in 0,1 N NaOH .

First derivative UV spectrophotometry (^1D)

There are two maxima (266.0 and 271.0 nm) and two minimum (240.0 and 307.2 nm) in the first derivative spectra of the solution of **ACE** in 0.1 N NaOH in the range of 230-325 nm (Figure 3). Different Δλ values were tested and Δλ= 2 nm was found optimal in the method. The determination of **ACE** can be realized by measuring the dA/dλ values at 240.0, 266.0 and 271.0 nm and using the calibration curve prepared by plotting the dA/dλ values versus ten different concentrations of standard substance. Linearity range according to the Beer's law was found as 8.0 – 85.0 μg/mL in the method. LOQ was 8.0 μg/mL and LOD was calculated as 2.66 μg/mL by using the following equation; 3.3 SD/m. Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 3.

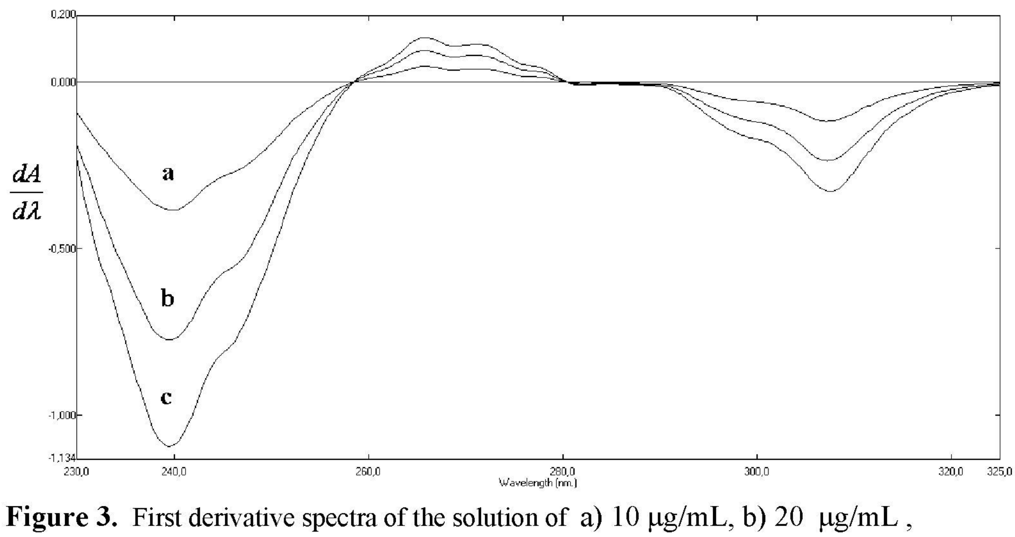

Figure 3. First derivative spectra of the solution of a) 10 μg/mL, b) 20 μg/mL , c) 30 μg/mL **ACE** in 0.1 N NaOH (Δλ = 2 nm) (Scaling factor = 10).

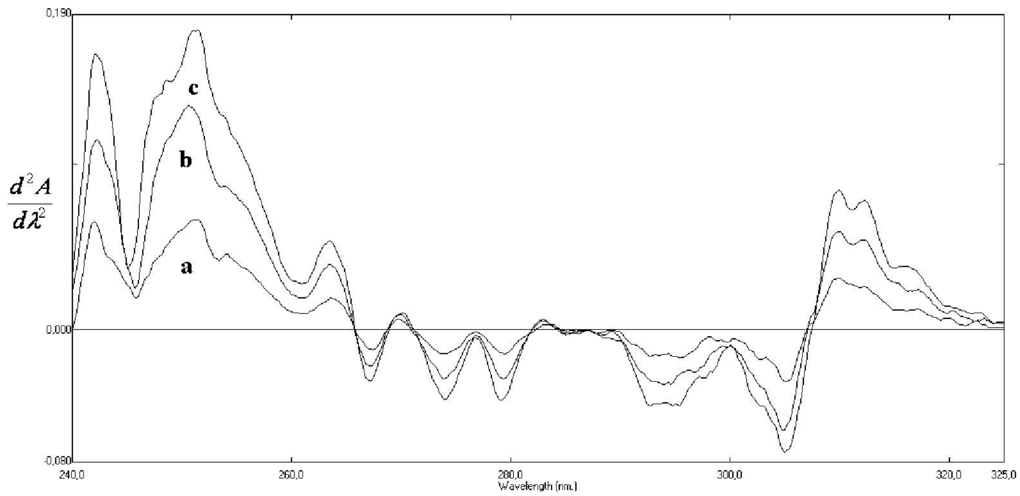

Figure 4. Second derivative spectra of the solution of a) 10 μg/mL, b) 20 μg/mL , c) 30 μg/mL **ACE** in 0.1 N NaOH ($\Delta\lambda$ = 2 nm) (Scaling factor = 20).

Second derivative UV spectrophotometry (^2D)

There are three maxima (249.2 and 298.4 nm) and four minima (264.0, 274.0, 279.6 and 304.8 nm) in the second derivative spectra of the solution of **ACE** in 0.1 N NaOH in the range of 240-325 nm (Figure 4). Different $\Delta\lambda$ values were tested and $\Delta\lambda$= 2 nm was found optimal in the method. The determination of acemetacin can be realized by measuring the $d^2A/d\lambda^2$ values at the wavelengths mentioned above and using the calibration curve prepared by plotting the $d^2A/d\lambda^2$ values versus ten different concentrations of standard substance. Linearity range according to the Beer's law was found as 15.0 – 85.0 μg/mL in the method. LOQ was 15.0 μg/mL and LOD was calculated as 5.0 μg/mL by using the following equation; 3.3 SD/m. Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 4.

Table 1. Regression parameters in spectrophotometric methods.

Methods	λ (nm)	m	n	r	Linear working ranges (µg/mL)
Original UV spektr.	225.0	0.0386	0.6882	0.8652	8-85
	280.0	0.0173	0.0041	0.9996	8-85
^{1}D	240.0	-0.0116	0.0026	0.9991	8-85
	266.0	0.0046	0.0007	0.9978	8-85
	271.0	0.004	-0.0008	0.9993	8-85
	307.2	-0.0162	-0.4981	0,9353	8-85
^{2}D	251.0	0.0063	0.0032	0.9937	15-85
	263.6	0.0019	-0.0018	0.9927	15-85
	267.0	-0.001	-0.0006	0.9872	15-85
	274.0	0.003	-0.0017	0.9965	15-85
	279.6	-0.0016	0.0042	0.9869	15-85
	304.8	-0.0029	0.0008	0.9913	15-85
	310.0	-0.0015	0.001	0.9881	15-85

m = scope, n = intercept, $y = mx + n$, r = correlation coefficient.

Table 2. Validation parameters in classical UV spectrophotometry using standard solutions of acemetacin in 0.1 N NaOH.

	280.0 nm		
No	**Added µg/mL**	**Found µg/mL**	**Recovery %**
1	8	8.14	101.81
2	15	15.08	100.54
3	25	24.96	99,84
4	35	35.54	101.55
5	64	63.40	99.06
6	72	72.48	100.66
7	85	85.86	101.01
n = 7		$\bar{X}$	100.64
		SD	0.96
		RSD	% 0.95

$\bar{X}$= mean, SD= Standard Deviation, RSD= Relative Standard Deviation

Table 3. Validation parameters in first derivative UV spectrophotometric method using standard solutions of acemetacin in 0.1 N NaOH.

		240.0 nm		266.0 nm		271.0 nm		307.2 nm	
	Added µg/mL	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**
1	8	7.99	99.90	8.76	109.51	8.95	111.86	8.11	101.33
2	15	15.09	100.60	15.93	106.23	16.45	109.67	15.08	100.52
3	25	24.83	99.28	23.97	95.91	24.45	97.80	24.78	99.10
4	35	35.06	100.17	36.80	105.15	36.70	104.86	35.45	101.28
5	64	64.75	101.17	62.46	97.59	60.95	95.23	63.35	98.99
6	72	72.50	100.70	73.98	102.75	74.70	103.75	72.38	100.53
7	85	85.95	100.70	88.18	103.74	86.76	102.07	85.21	100.25
n = 7		$\bar{X}$	100.36		102.98		103.60		100.29
		SD	0.62		4.79		5.95		0.94
		RSD	% 0.62		% 4.65		% 5.75		% 0.94

Table 4. Validation parameters in second derivative UV spectrophotometric method using standard solutions of acemetacin in 0.1 N NaOH

Added (µg/mL)	251.0 nm		263.6 nm		267.0 nm		274.0 nm		279.6 nm		304.8 nm		310.0 nm	
	Found (µg/mL)	**Recovery %**	**Found (µg/mL)**	**Recovery %**	**Found (µg/mL)**	**Recovery %**	**Found (µg/mL)**	**Recovery %**	**Found (µg/mL)**	**Recovery %**	**Found (µg/mL)**	**Recovery %**	**Bulunan (µg/mL)**	**% Geri Kazanım**
15	15.60	104.06	16.21	108.07	15.60	104.00	15.00	100.00	15.40	102.66	15.05	100.33	15.17	101.10
25	24.35	97.44	23.05	92.21	22.60	90.40	24.64	98.57	24.73	98.93	24.38	97.52	25.30	101.20
35	34.67	99.06	33.05	94.43	34.60	98.85	35.35	101.02	34.73	99.23	34.88	99.64	35.70	102.00
64	60.76	94.95	59.36	92.76	65.60	102.50	65.17	101.83	65.40	102.18	63.58	99.34	63.36	99.00
72	70.30	97.63	71.47	99.26	70.60	98.05	72.50	100.69	70.73	98.24	73.05	101.46	70.63	98.10
85	85.99	101.16	82.82	97.43	92.87	109.26	85.51	100.60	75.39	88.69	82.81	97.42	86.69	101.99
	$\bar{X}$	99.05		97.36		100.51		100.45		98.32		99.29		100.57
	SD	3.19		5.90		6.38		1.10		5.05		1.59		1.63
	RSD	%3.22		% 6.05		% 6.34		% 1.10		% 5.13		% 1.86		%1.62

High performance liquid chromatographic analysis

An new isocratic programme was developed for optimal separation and determination of **ACE.** In the method**,** ACE C18 analytical column and mobile phase composed of acetonitrile – water (80:20, v/v) at a flow rate of 1.0 mL/min and detection at 280 nm were found for the optimal chromatographic separation. Dienogest **(DNG)** was selected as internal standard. Under the chromatographic conditions employed, **ACE** and **DNG** were well resolved and their retention times were found to be 1.56 and 3.15 min, respectively. A typical chromatogram of the drugs and internal standard was illustrated in Figure 5. The values of suitability test are in the range of expected values which means that HPLC method used in this study is appropriate for the measurement of concentration of **ACE** using **DNG** as internal standard.

The calibration curves were established with ten different concentrations of **ACE** in the range of 5.0 - 100.0 μg/mL. A triplicate injection was carried out from each standard solution and the peak areas were measured at 280 nm. The ratios of the peak areas of investigated substances to that of internal standard were calculated for each injection. Regression equation was established by plotting the ratio of peak areas to the concentration of **ACE**. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression method. Regression equation was;

$y = 0.1703\ x + 0.0207 \quad (r = 0.9999)$

where x is the concentration of **ACE** as μg/mL, y is the ratio of peak areas.

LOQ was 5.0 μg/mL and LOD was calculated as 0.07 μg/mL by using the following equation; 3.3 SD/m (SD=Standard deviation, m=slope)

Mean recoveries and relative standard deviations calculated for standard solutions were shown in Table 5. Statistical values in the table indicate that the method is appropriate for determination of **ACE** with optimum recovery.

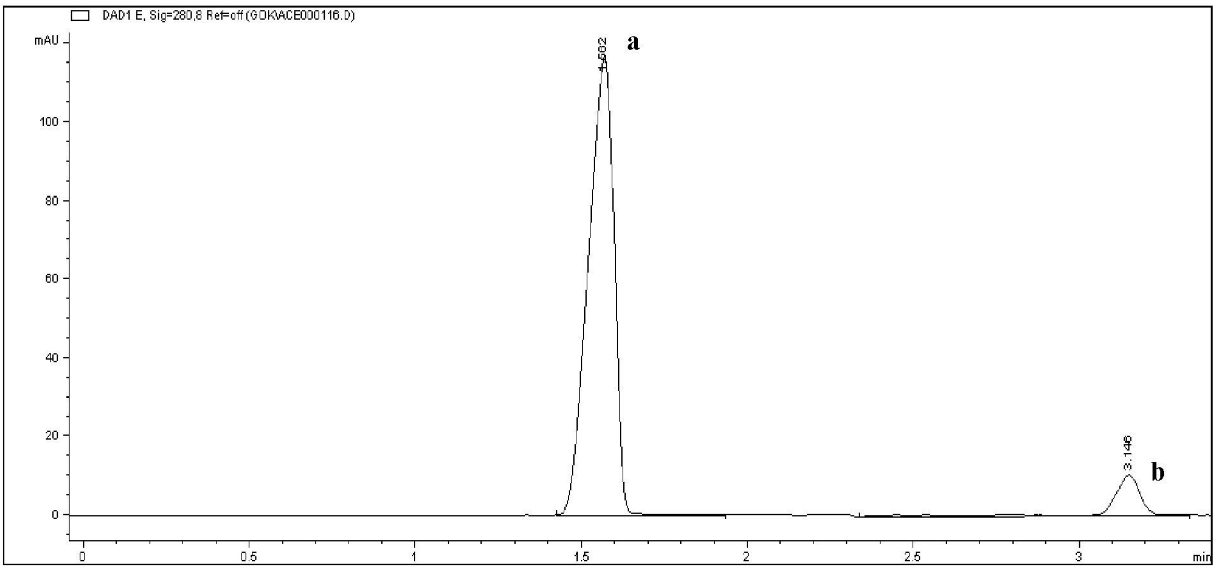

Figure 5. Chromatogram of the solution of a) 75 μg/mL **ACE** and b) 5 μg/mL **dienogest** (IS) in acetonitrile.

Table 5 . Validation parameters in HPLC using standard solutions of acemetacin in acetonitrile.

No	Added µg/mL	Found µg/mL	Recovery %
1	30	29.88	99.59
2	30	30.20	100.65
3	30	30.26	100.85
4	55	55.17	100.30
5	55	55.35	100.63
6	55	55.47	100.86
7	75	74.97	99.96
8	75	74.47	99.29
9	75	75.63	100.84
		$\bar{X}$	100.33
		SD	0.59
n = 9		RSD	% 0.59

Selectivity

According to official validation guidelines, in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason, in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis, the recovery test was done by the standard addition method by adding known amounts of **ACE** at three different concentrations corresponding to 1/3, 2/3 and 3/3 of the label claims. Each solution was prepared in triplicate and the methods were applied. According to the recoveries obtained for the amount of the added **ACE** (100.14 – 101.47 % for all the formulations selected) when applied three methods at selected wavelengths (at 280.0 nm in original UV spectrophotometry, at 240.0 nm in first derivative UV spectrophotometry and at 274.0 nm in second derivative UV spectrophotometry) and HPLC method. It was concluded that there was no interference from the ingredients placed in the formulations.

Accuracy and Precision

Accuracy in the methods was determined by the recovery studies using standard solutions of **ACE.** In original UV spectrophotometry**:** the mean recovery and relative standart deviation were found as 100.64 and 0.95 % at 280.0 nm (Table 2). In first derivative UV spectrophotometric method; the mean recoveries were found as 100.36, 102.98, 103.60 and 100.29 % at 240.0, 266.0, 271.0 and 307.2 nm respectively. Relative standard deviations at these wavelengths were found as 0.62, 4.79, 5.95 and 0.94 % respectively (Table 3). In second derivative UV spectrophotometric method; the mean recoveries were found as 99.05, 97.36, 100.51, 100.45, 98.32, 99.29 and 100.57 % at 251.0, 263.6, 267.0, 274.0, 279.6, 304.8 and 310.0 nm respectively. Relative standard deviations at these wavelengths were found as 3.22, 6.05, 6.34, 1.10, 5.13, 1.86 and 1.62 % (Table 4). In HPLC, the mean recovery and relative standart deviation were 100.33 % and 0.59 % respectively (Table 5).

Robustness

Robustness was tested by changing the concentration of NaOH. No significant difference was observed for 0.05 – 0.15 N NaOH range. We selected 0.1N NaOH for the spectrophotometric methods proposed.

Solution Stability

Solution of **ACE** in 0.1N NaOH is stable for 48 hours at room temperature.

Analysis of Pharmaceutical Preparations

Developed four methods were applied to the determination of **ACE** in pharmaceutical preparations selected. Each pharmaceutical preparation was analyzed by performing ten independent determinations. In application, 280.0 nm in original UV spectrophotometry, 240.0 nm in first derivative spectrophotometry and 274.0 nm in second derivative spectrophotometry were selected by their lowest RSD values in the validation studies, Table 2-4. Satisfactory results were obtained for **ACE** and were found to be in agreement with the label claims (Table 6). The results obtained by the developed methods were compared with a HPLC method statistically by using Student's *t* test and no significant difference was observed between them by the fact that *t* values calculated were lower than that of tabulated (theoretical) values for p = 0.05 level (Table 7). HPLC method that is used for comparison was developed by us.

Table 6. Assay results of commercial formulations for **ACE.**

Methods / **Pharmaceutical preparatons**	**^{1}D** Mean (mg) ± SD (% RSD)	**^{2}D** Mean (mg) ± SD (% RSD)	**Original UV Spectroscopy** Mean (mg) ± SD (% RSD)	**HPLC** Mean (mg) ± SD (% RSD)
RANTUDİL Forte (60 mg)	62.35 ± 1.02 (% 1.64)	63.02 ± 2.26 (% 3.59)	62.33 ± 1.09 (% 1.76)	61.59 ± 0.53 (%0.87)
RANTUDİL Retard (90 mg)	90.51 ± 0.57 (% 0.64)	90.81 ± 2.89 (%3.18)	90.53 ± 1.22 (% 1.35)	90.01 ± 1.82 (% 2.03)

* Mean of ten replicates
** SD = Standard deviation,
*** RSD = Relative Standard deviation

Table 7. Calculated *t* values when compared the results with those obtained by HPLC method developed by us.

	UV – ^{1}D	UV – ^{2}D	^{2}D – ^{1}D	UV- HPLC	^{1}D - HPLC	^{2}D - HPLC
RANTUDİL Forte (60 mg)	0.04	0.87	0.85	1.91	2.10	1.95
RANTUDİL Retard (90 mg)	0.05	1.22	0.32	0.74	0.83	0.73

*Tabulated value of *t* is 2.26 for p = 0.05

CONCLUSION

Three spectrophotometric methods, original UV spectrophotometry and, first and second derivative UV spectrophotometry were developed and they were succesfully applied to the determination of **ACE** in 2 different pharmaceutical formulations after their optimization and validation. Proposed spectrophotometric methods are original and very simple methods for the determination of **ACE** in pharmaceutical preparations. In addition an HPLC method was developed for comparision of the results. In literatures, it is seen that two HPLC methods developed for the determination of **ACE** but these methods are very difficult and the materials used are not simply findeble. These four methods were found accurate and precise and, applicable for the routine analysis of the formulations. Good agreement was achieved in the assay results of pharmaceutical preparations, tablets, for three spectrophotometric methods proposed in the text. So, these methods can be apply accurately and precisely for the analysis of **ACE** in the pharmaceutical preparations, tablets, without prior separation procedure in place of HPLC method which is tedious, time consuming and expensive method.

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