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RESEARCH ARTICLES / BILIMSEL ARAŞTIRMALAR

Quantitative Determination of Aspirin and Paracetamol in Tablets

Okan Atay*, Figen Dinçol**

Summary: Quantitative analysis of aspirin (ASP) and paracetamol (PA) containing tablets, which are widely used for analgesic-antipyretic purposes in this country, was carried out by employing various analytical methods in this study. Two different spectrophotometric, and a high pressure liquid chromatography(HPLC) methods have been proposed for this combination.

In the spectrophotometric absorbance ratio method, the maximum absorbance at 276 and 243 nm ASP + PA synthetic mixture in 0.1 N HCl were utilized and 231 nm was chosen as the isosbestic point. The absorbance ratios, 276/231 and 243/231, have been used in the regression analysis.

In the Vierordt method, A1(%1,1 cm) values of the two active principles have been determined at 276 and 243 nm in 0.1 N HCl, and the values of $a = \alpha_2/\alpha_1$ and $b = \beta_2/\beta_1$ were found by using A1 values The a and b coefficients have been applied to the equation, which was developed by Vierordt.

In the high pressure liquid chromatography method, different working conditions from the monograph in USP XXII were applied and sodium benzoate was used as the internal standard.

For the quantitative determination, different regression equations were utilized for each method. In the absorbance ratio, Vierordt and HPLC methods, relative standard deviations were found as 0.89, 1, 1.4 % for ASP and 1.24, 1.39, 0.84 % for PA respectively.

Keywords : Aspirin, paracetamol, absorbance, ratio, Vierordt, HPLC methods

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Aspirin ve Parasetamol İçeren Tabletlerde Miktar Tayini Çalışmaları

Özet: Bu çalışmada ağrı kesici ve ateş düşürücü etkileri nedeni ile ülkemizde de sıklıkla kullanılan aspirin (ASP)-Paracetamol (PA) içeren tabletlere değişik analiz yöntemleri uygulanarak miktar tayini çalışmaları yapılmıştır. Bu kombinasyon için farklı iki spektrofotometrik ve yüksek basınçlı sıvı kromatografisi yöntemi önerilmiştir.

Absorbans oranları yönteminde, ASP+PA sentetik karışımlarının sıra ile 0.1 N HCl de 276 ve 243 nm'deki maksimum absorbans değerlerinden yararlanılmış, izosbestlik nokta olarak 231 nm seçilmiştir. Regresyon denklemlerinin oluşumunda 276/231 ve 243/231 absorbans oranları kullanılmıştır.

Virerordt yönteminde her iki etken maddenin 0.1 N HCl'de 276 ve 243 nm'deki A1(:%1,1 cm) değerleri hesaplanmış, bu değerlerden yararlanılarak bulunan a ve b katsayıları Vierordot'un geliştirdiği formüllere uygulanmıştır. Yüksek basınçlı sıvı kromatografi(YBSK) yöntemi ile yapılan çalışmalarda ise, USP XXII'deki monografi yönteminden farklı çalışma koşulları uygulanmış ve iç standart olarak sodyum benzoat kullanılmıştır.

Her yöntem için ayrı ayrı oluşturulan regresyon denklemlerinden kantitatif tayinler için yararlanılmıştır.

Absorbans oranları, Vierordt ve YBSK yöntemlerinde yüzde bağıl standart sapma ASP için sıra ile % 0.89, 1, 1.41, PA için % 1.24, 1.39, 0.84 olarak bulunmuştur.

Anahtar sözcükler : Aspirin, parasetamol, absorbans oranları yöntemi, Vierordt yöntemi, YBSK yöntemi

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Introduction

ASP and PA are used for analgesic and antipyretic purposes. They are used alone or in combination with different compounds. The combination of ASP and PA are among the most consumed ones in our country, since they are effective drugs and have a wide therapeutic index.

The procedure described in this article is provided for the quantitative determination of ASP and PA in

(*) Faculty of Pharmacy of Gazi University, Ankara.
(**) Refik Saydam Hygiene Institute Drug and Cosmetic Research Laboratory, Ankara.

commercial tablets, without any seperation processes.

According to previous studies, the quantitative determination of ASP and PA one at a time in different pharmaceutical dosage forms and body fluids has been carried out by titrimetry[1], visible spectroscopy[2,3,4], UV spectroscopy[5,6,7,8] derivative spectroscopy[9,10,11,12], fluorometry[13], NMR spectroscopy[14] liquid chromatography[15] and HPLC[16,17,18].

Spectrophotometric methods(absorbance ratio and Vierordt) were carried out for ASP + PA combination for the first time.

Materials and Methods

In spectrophotometric studies, a Shimadzu UV 160-A model recording spectrophotometer was utilized.

IN HPLC studies, Shimadzu LC-6A pump, SPD-6AV detector, SCL-6A system controller and CR3-A integrator were used.

ASP and PA were obtained from Bayer and Atabay Drug companies. All solvents and chemicals used were reagent grade. The commercial pharmaceutical preparations with different serial numbers were purchased from local pharmacies in Ankara.

Absorbance Ratio Method:

Synthetic mixtures, containing ASP and PA at different concentrations were prepared and asorbance values were measured at 276, 243 and 231 nm(isosbestic point) in 0.1 HCl (Figure 1). Regression analyses were done for each active substance. In these equations, X value stands for the ratio of one active substance to the sum of two active substances and y value stands for the absorbance ratios 276/231 for ASP and 243/231 for PA.

Vierordt's Method

A1 values(%1,1 cm) of ASP and PA were found at 276 and 243 nm in 0.1 N HCl, a ve b coefficients were determined using these aborbance values. The coefficients and total absorbance values were put into the equation and active substance concentrations were calculated.

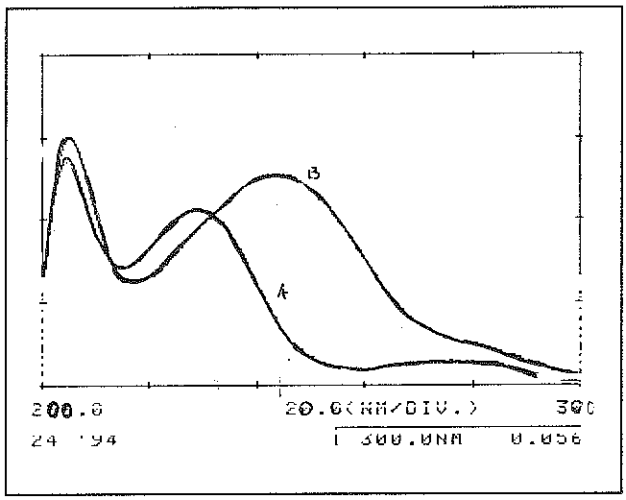

Figure 1. Zero order spectra of aspirin (A) and paracetamol (B). Aspirin and paracetamol concentrations are 20 µg/mL in 0.1 N HCl.

High-Pressure-Liquid Chromatography(HPLC)

In this method, working conditions were different than those of the monograph in USP XXI. Sodium benzoate(SB), was used as the internal standard. In the regression equations, for the X values, PA/SB and ASP/SB concentration ratios were used, whereas peak area ratios of PA/SB and ASP/SB were used for the Y values. (Figure-2)

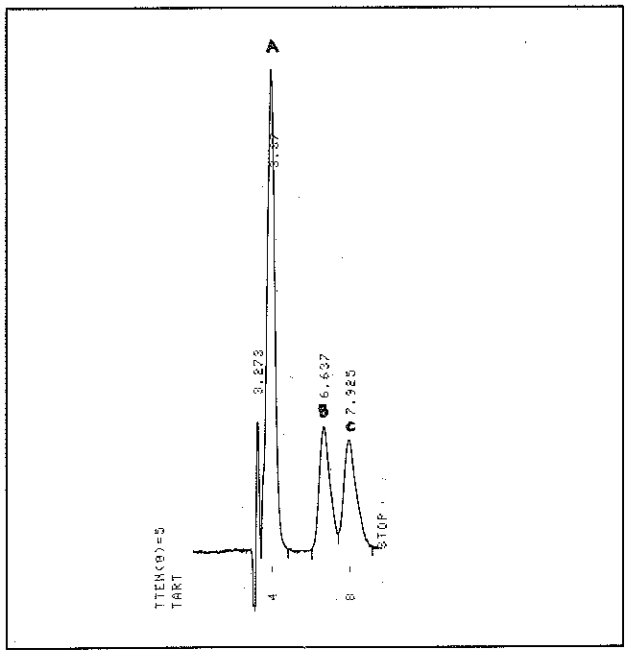

Figure 2. Chromatogram of paracetamol(A), aspirin(B), sodium benzoate(C) in the HPLC method. Aspirin and sodium benzoat are 0,4 mg/mL and paracetamol is 0,8 mg/mL in mixture solution (Injected volume is 20 µL).

In this method, the following operating conditions were applied:
Column: Hypersil BOS C_{18} 5 µm (250 x 4 mm)
Mobil phase: Water-methanol-glacial acetic acid (960:280:30)
Solvent mixture: Methanol-glacial acetic acid(95:5)
Detector: UV 275 nm
Flow-rate: 2 mL/min
Int. Standard : Sodium benzoate

Preparation of the Synthetic Mixtures

Absorbance ratio method
Stock ASP solution: 500 µg/mL ASP in 0.1 N HCl.
Stock PA solution: 500 µg/mL PA in 0.1 N HCl.

The absorbances of the synthetic mixtures, which were prepared by taking the portions from the stock solutions at the volumes determined in Table-1 were measured at 276, 243 and 231 nm and regression equations were established for ASP and PA.

Vierordt's method

1, 2, 3, 4, and 5 mL portions were taken from the stock ASP solution and put into 50 mL volumetric flasks and diluted with 0.1 N HCl. The absorbances of the solution were measured at 276 and 243 nm separately, using 0.1 N HCl as the reference.

1, 2, 3, 4 and 5 mL portions were taken from stock PA solution and put into 50 mL of volumetric flasks and diluted with 0.1 N HCl. The aborbances of the solutions prepared were measured separetely at 276 nm using 0.1 N HCl as the reference.

A 10 mL portion was taken from stock PA solution and diluted with 0.1 N HCl to 50 mL and 1, 2, 3, 4 ad 5 mL portions were taken from this solution and diluted with 0.1 N HCl to 50 mL. The absorbance of solutions were measured separately at 243 nm using 0.1 N HCl as the reference.

The A1 values of ASP and PA were calculated from the measured absorbance values. These values are shown in Table-2.

High Pressure Liquid Chromatography Method

Stock Solutions:
ASP, PA and SB solutions, which contain 5 mg/mL active substances were prepared separately with the solvent mixture.

Table 1. Results of the Regression Equation in the Absorbance Ratio Method

Synthetic Mixtures*				Ratio of the synthetic mixture		Absorbance Ratio	
mL		µg					
Stock ASP. sol.	Stock PA. Sol.	ASP	PA	ASP	PA	ASP 276/231	PA 243/231
1	1	10	10	0.5	0.5	0.212	0.867
1	1.5	10	15	0.4	0.6	0.226	0.975
1.5	1	15	10	0.6	0.4	0.197	0.796
2	1	20	10	0.666	0.333	0.175	0.707
2.5	1.5	25	15	0.625	0.375	0.187	0.771

	Slope (a)	Intercept (b)	Coeff. of determination	Regression Equation
ASP	-0.145	0.284	0.999	y = - 0.145 x + 0.284
PA	1.00	0.372	0.999	y = 1.00 x + 0.372

* Mixtures were diluted to 50 mL with 0.1 N HCl

Table 2. A1 Values in Vierordt's Method

α_1	α_2	$ß_1$	$ß_2$	$\alpha=\alpha_2/\alpha_1$	$b=ß_2/ß_1$	m
643	132	166	64,5	0,204	0,389	A_2/A_1

α_1 = Paracetamol 243 nm A^1 value in 0.1 N HCl
α_2 = Paracetamol 276 nm A^1 value in 0.1 N HCl
$ß_1$ = Aspirin 243 nm A^1 value in 0.1 N HCl
$ß_2$ = Aspirin 276 nm A^1 value in 0.1 N HCl
A_1 = Total absorbance at 243 nm.
A_2 = Total absorbance at 276 nm.

Different volumes of solution were taken from each stock solution as shown in Table-3 and synthetic mixtures of different concentrations were obtained. 20 µL was injected on to the column from these mixtres. Three separate injections were done for each solution. The average value of peak areas obtained by the integrator were used. Regression equations and the values found are shown in Table-4.

Table 3. Synthetic Mixtures in the HPLC method.

	Stock Solution (mL)			Active substance in injected volume (µg)		
	ASP	PA	SB	ASP	PA	SB
ST1	1	1	1	4	4	4
ST2	1,5	1,5	1	6	6	4
ST3	2,5	2,5	1	10	10	4
ST4	1	2	1	4	8	4
ST5	2	1	1	8	4	4

* Standard mixtures were diluted to 25 mL in MeOH-Glacial acetic acid (95:5)

Results obtained for the analysis of synthetic mixtures by the proposed methods are shown in Table-5.

Preparation of Samples:

Absorbance ratio and Vierord'ts methods

Twenty tablets were weighed, powdered and mixed homogenously. The powder was accurately weighed, which was equivalent to 25 mg ASP and PA. It was extracted for ten minutes with metanol and then filtered. A 2 mL portion was taken from the filtrate, and diluted to 100 mL with 0.1 N HCl. The absorbances were measured at 276, 243 and 231 nm in the spectrophotometric methods.

HPLC Method

The powder obtained as mentioned above was accurately weighed, which was equivalent to 25 mg ASP and PA, and extracted with 30 mL of solvent mixture and filtered. A 3 mL internal standard solution was added and scaled up to 50 mL with solvent mixture. Then, 15 µL was injected.

Calculation

Absorbance ratio method

The concentration of substances were calculated from the equation shown below:

$$C=\frac{Q-b}{a}x\frac{A_{ISO}}{A_{ISO}}x\,10^{3}=\mu g/mL$$

In this equation,

Q = The ratio of absorbance values, which was measured from the sample. It is 243/231 for PA and 276/231 for ASP.

a and b are the slope and intercept values for ASP and PA respectively(Table-1).

A_{ISO} = Absorbance value of the sample at 231 nm (isosbestic point)

a_{ISO} = Absorptivity value at 231 nm. (In this study, absorptivity value was found to be 45, 9).

Vierordt's Method

The concentration of the substances were calculated from the equations shown below:

$$PA=\frac{A_1}{\alpha_1 x\,10^{-3}}x\frac{b-m}{b-m}$$

$$ASP=\frac{A_2}{\alpha_2 x\,10^{-3}}x\frac{b(m-a)}{m(b-a)}$$

(Concentration of the substances were found in mg/100 mL.)

HPLC Method:

The peak area ratios ASP/SB and PA/SB, which were established previously for ASP, PA and SB, were put into regression equation shown in Table-4 and the quantity of active substances were calculated.

Results obtained for the analysis of commercial tablets are shown in Table-6.

Table 4. Concentration and Peak Area Ratios and Regression Equations of the HPLC Method

Synthetic mixture				
	ASP		PA	
	Conc. Ratio	Peak Area Ratio	Conc. Ratio	Peak Area Ratio
	ASP/SB	ASP/SB	PA/SB	PA/SB
	1	0.9336	1	2.832
	1,5	1.447	1,5	4.134
	2,5	2.387	2,5	6.698
	1	0.941	2	5.533
	2	1.922	1	2.870
y = ax+b	y = 0.970 x - 0,025		y = 2.60 x + 0.265	
Coeff. of determ.	0.999		0.999	

Table 5. Results Obtained for the Analysis of Synthetic Mixtures by the Proposed Methods

Synthetic mixtures µg/mL		Found µg/mL					
		Absorbance ratio method		Vierordt method		HPLC method	
ASP	PA	ASP	PA	ASP	PA	ASP	PA
10	10	9.8	9.9	10.1	9.8		
10	15	9.8	15.0	9.7	15.2		
15	10	14.7	10.1	14.8	10.1		
20	10	19.8	10.2	19.6	10.2		
4	4					3.9	3.9
6	6					6.1	6
4	8					4	8.2
8	4					8.1	4.0

Table 6. Results Obtained for the Analysis of Commercial Tablets

ASP 250 mg PA 250 mg	Absorbance Ratio Method		Vierordt Method		HPLC Method	
	ASP	PA	ASP	PA	ASP	PA
	245	249	252	247	244	252
	250	249	245	253	246	248
	247	253	248	252	252	247
	253	252	250	249	250	250
$\bar{X}$	249	250	248	249	247	249
St Deviation	2	3	3	3	3	2
Rel. St. Dev %	0.89	1.24	1.1	1.39	1.41	0.84
p = 0.05 Confidence Interval	246-251	247-254	245-251	245-254	243-252	247-252

Results and Discussion

The procedure described in this paper is provided for the quantitative determination of ASP and PA in commercal tablets.

The proposed methods are practical, accurate and precise. The suggested spectrophotometric methods(absorbance ratio and Vierordt) assay of ASP or PA with tablets it can be carried out without any separation process, for the ratios of ASP and PA 1:1, 2:1, 1:2, respectively.

In the HPLC method, different working conditions were established and a different internal standard than that of USP XXII was used.

The relative standard deviations for the absorbance ratio, Vierordt and HPLC methods were found to be 0,89, 1,1 and 1,41 % for ASP and 1,24, 1,39, 0,84 % for PA respectively.

The precision of the three methods were compared using Students' t and Fisher F tets. As can be seen in Table 7, the absorbance ratio and Vierordt methods are not significantly different from the HPLC method, and the three methods have comparable precision.

The specificity of each method for ASP or PA was tested by preparing calibration graphs using synthet-

ic mixtures prepared by standard, where the three methods all showed a linear relationship with acceptable coefficients of determination (Table 1, 2, 4).

Table 7. Comparision of the Results Obtained for the Precision in the Proposed Method.

Student t test	
p = 0.05	
n = 10-2 = 8 t table value 1,86	
ASPIRIN	
Absorbance ratio - Vierordt	0.213 < 1.86 Not significant
Absorbance ratio - HPLC	0.619 < 1.86 Not significant
Vierordt - HPLC	0.406 < 1..86 Not significant
PARACETAMOL	
Absorbance ratio - Vierordt	0.633 < 1.86 Not significant
Absorbance ratio - HPLC	0.730 < 1.86 Not significant
Vierordt - HPLC	0.384 < 1.86 Not significant
Fisher Test (F Test)	
p = 0.05	
n = 5-1 = 4	
F table value 6.39	
ASPIRIN	
Absorbance ratio - Vierordt	1.46 < 6.39 Not significant
Absorbance ratio - HPLC	2.45 < 6.39 Not significant
Vierordt - Vierordt	1.68 < 6.39 Not significant
PARACETAMOL	
Absorbance ratio - Vierordt	1.24 < 6.39 Not significant
Absorbance ratio - HPLC	2.18 < 6.39 Not significant
Vierordt - HPLC	2.7 < 6.39 Not significant

The results, which we have obtained from spectrophotometric methods, have shown that, they can be an alternative to the HPLC method with respect to precision and accuracy. Also, these methods can be used in dissolution tests of ASP and PA tablets. The results of this study will be published in another paper.

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