Applied Chemometric Method Based on H-Point Standard Addition Method (HPSAM) for Simultaneous Spectrophotometric Estimation of Binary Mixture

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A new simultaneous spectrophotometric approach for determining mefenamic acid (Mef) and piroxicam (Pir) was developed using the H-point standard addition method (HPSAM). The addition of standard mefenamic acid solutions was monitored by measuring absorbance at 305.3 and 343.1 nm. The study found that mefenamic acid and piroxicam can be detected simultaneously in prepared combinations at concentration ratios ranging from 2:4 to 10:6 μg mL^{-1}. The limit of detection LOD was found to be 0.007 μg mL^{-1} for Mef and Pir. The percentage recovery ranged from 96.9 to 108.5% for mefenamic acid and 93.9 to 111.3% for piroxicam, with satisfactory precision. The proposed method was effectively used to determine Mef and Pir in both pure and commercial formulations.

Keywords: chemometric, H-point standard addition method, mefenamic acid, piroxicam

Introduction

NSAIDs, or nonsteroidal anti-inflammatory medications, are commonly used to treat pain, fever, and inflammation. They are among the most often utilized medical specialties. An estimated 30 million or more people receive NSAIDs every day worldwide [1] Mefenamic acid is a member of the NSAID (fenamate) class of anthranilic acid derivatives. Mefenamic acid, like other NSAIDs, inhibits cyclo-oxygenase (Cox-1 and -2) and prevents the synthesis of intracellular prostaglandins, which are crucial for pain and inflammation pathways. Although having analgesic, antipyretic, and anti-inflammatory properties, mefenamic acid is mostly utilized to treat pain. Despite being licensed in the US in 1967, mefenamic acid is not a widely used medication. The oxicam family, a class of enolic acids chemically distinct from other NSAIDs, includes piroxicam. Similar to other nonsteroidal anti-inflammatory drugs (NSAIDs), piroxicam works by inhibiting tissue cyclooxygenases (Cox-1 and -2), which reduces the production of pro-inflammatory prostaglandins, an important mediator of pain and inflammation. Along with its antipyretic and anti-inflammatory properties, piroxicam also possesses analgesic properties. Since its approval for usage in the US in 1982, piroxicam has been used extensively, with millions of prescriptions being filled each year [2]. The structural formulae for mefenamic acid and piroxicam are shown in Figure 1 [3].

Several common analytical techniques for the simultaneous or individual quantification of pharmacological formulations are revealed by a review of the literature. These methods include flow injection analysis [4-6] GC-mass [7-8], potentiometry [9-10], spectrophotometry [11-13], chemometric technique [14], HPLC [15-18] and artificial neural network ANN [19].

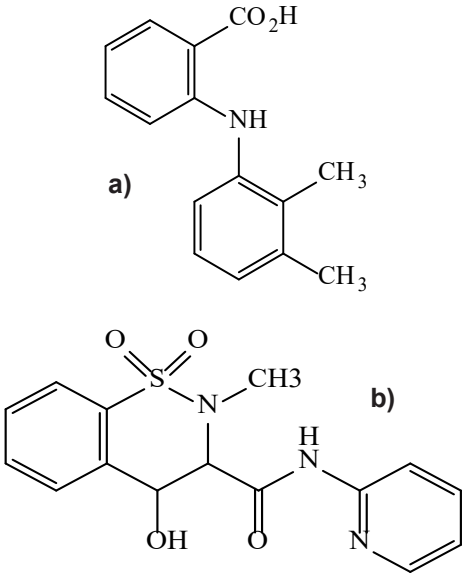

Figure 1. Structural formulas of (a) mefenamic acid and (b) piroxicam.

As far as we know, there isn't a documented procedure for utilizing the H-point assay technique to determine this drug combo. This technique differs from other chemometrics methods that require mathematical pretreatment, extensive wavelength selection, which increase the method complexity. While, H-point offers simplicity of operation without

need building complex model, accurately, quickly, and easily determine the combination of Mef and Pir in pharmaceutical formulations without being influenced by the additives or excipients.

Experimental part

Apparatus

UV-Vis spectra were acquired using a Shimadzu 1800 UV-Visible spectrophotometer (Kyoto, Japan) fitted with 1 cm matched cells. The Sartorius BL 210S balance.

Reagents

All chemicals used in the studies were analytical reagent grade. Samara Company of Iraq (SDI) provided mefenamic acid and piroxicam. Pharmaceutical that has been utilized in applications, Mefenamic acid syrup Ponamec (MVC-India) 50 mg/5mL and Feldene as solution for injection IM (Pfizer-France) labeled to contain 20 mg mL^{-1}. Both were obtained at local pharmacies. A standard solution of Mef and Pir (500 µg mL^{-1}) were prepared by dissolving accurately 0.05 g in sufficient amount of HPLC grade acetonitrile (Merck, Germany) and diluted by the same solvent in 100 mL volumetric flask. Several solutions were prepared from the stock solution and diluted by acetonitrile solvent.

Pharmaceutical preparation

Mefenamic acid and Piroxicam were prepared by withdrawing 0.5 mL and 0.25 mL for Mefenamic acid syrup and Feldene ampoule, respectively. Then transferring it to a volumetric flask of 50 mL, diluting it with acetonitrile to the mark to get 100 µg mL^{-1}. From these solutions several concentrations were prepared by the same solvent.

Laboratory prepared mixtures

Aliquots from each of the standard solutions were transferred to volumetric flasks (5 mL) in order to perform different ratios of Mef and Pir. Acetonitrile was then used for dilution.

Construction of individual calibration curve

To know the measured sample range for Mef and Pir, it is important to construct the individual calibration curve for each drug. Aliquots of Mef and Pir working standard solution (100 µg.mL^{-1}) (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mL) were added to 5 mL volumetric flasks, and acetonitrile was then diluted to create working solutions equal to 1–10 µg mL^{-1}. The absorption spectra were measured across the wavelength range of 200–400 nm at room temperature.

Recommended method

5-milliliter volumetric flasks were filled with different concentrations ratio of Mef and Pir by adding (0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.45 and 0.5 mL) of Mef and (0.1, 0.2, 0.3, 0.4 and 0.5 mL) of Pir, then standard addition of Mefenamic acid (up to 8.0 µg mL^{-1}) was made to synthetic samples and diluted to the mark with acetonitrile. Measurements of the absorbance at suitable wavelengths (305.3 and 343.1 nm) were made by transferring a portion of the solution into a 1 cm quartz cell. The concentration range for Mef and Pir that used to demonstrate the applicability of HPSAM were 2-10 µg mL^{-1} for both drugs.

H-point standard addition method

Imagine a sample that is unknown and contains the interferent Y and the analyte X. Mefenamic acid was regarded as the analyte in this particular system, while piroxicam was considered the interferent. In these circumstances, the choice of two wavelengths λ1 and λ2 where the interferent species, Y, exhibits identical absorbance is necessary for the HPSAM method of determining the concentration of X. Following the addition of known volumes of X to the mixture, the absorbances at the two wavelengths are measured and expressed using the following equations:

$$A_{(\lambda 1)} = b_o + b + M_{\lambda 1} C_i \quad \text{Eq. 1}$$

$$A_{(\lambda 2)} = A_o + A + M_{\lambda 2} C_i \quad \text{Eq. 2}$$

where:

$A_{(\lambda 1)}$, $A_{(\lambda 2)}$: The analytical signals measured at λ1 and λ2.
b_o, A_o: The original analytical signals of analyte X at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$, ($b_o \neq A_o$).
b, A: The analytical signals of interferent Y at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$
$M_{\lambda 1}$, $M_{\lambda 2}$: The slopes of the standard addition calibration lines at λ1 and λ2.
C_i: The added X concentration.
($-C_H$, A_H): The intersection of the two straight lines, known as the H-point.

At H-point, since $A_{(\lambda 1)} = A_{(\lambda 2)}$, $C_i = C_H$ from equations 1 and 2 it follows that:

$$b_o + b + M_{\lambda 1}(-C_H) = A_o + A + M_{\lambda 2}(-C_H) \quad \text{Eq. 3}$$

$$-C_H = [(A_o - b_o) + (A - b)] / (M_{\lambda 1} - M_{\lambda 2}) \quad \text{Eq. 4}$$

According to equation 4, if Y is the known interferent and b (at λ1 or λ2) is the analytical signal corresponding to Y, then b = A = constant and does not change when analyte X is added.

$$-C_H = (A_o - b_o)/(M_{\lambda 1} - M_{\lambda 2}) = -b_o/M_{\lambda 1} = -A_o/M_{\lambda 2} \quad \text{Eq. 5}$$

where $C_H = C_X$ and C_X indicates the amount of analyte present in the mixture, because $-C_H$ exclusively depends on analyte-related variables [20].

Should the value of $-C_H$ be incorporated into equation 1, A_H, the coordinate value of the crossing point will be explicated as follows:

$$A_H = b_o + b + M_{\lambda 1}(-C_H) \quad \text{Eq. 6}$$

As $b_o = M_{\lambda 1} C_H$, then $A_H = b$ and similarly,

$$A_H = A \quad \text{Eq. 7}$$

As a result, the A_H value only has a relationship with the interferent Y signal at the two chosen wavelengths. A calibration graph is necessary in order to calculate the interference concentration from the ordinate value of the H-point (A_H).

Results and discussion

Absorption spectra and wavelength selection

Figure 2 displays the absorption spectra of Mefenamic acid and Piroxicam. Figure 2 illustrates the near proximity of two compounds' maximum

wavelengths and the significant overlap in their spectra. Consequently, it is not possible to determine Mefenamic acid and Piroxicam in combination using traditional spectrophotometry. For this reason, a chemometrics approach must be used to solve this issue.

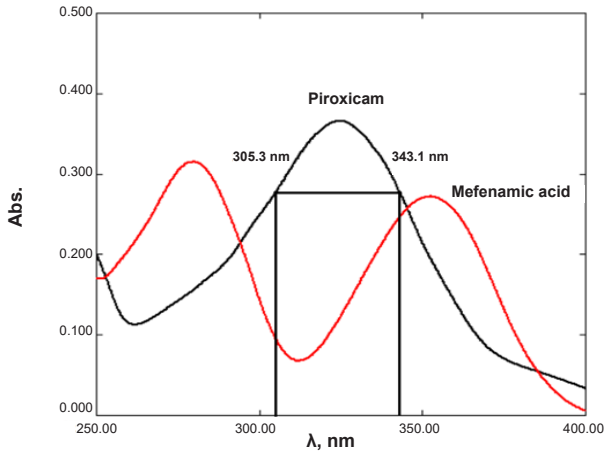

Figure 2. Absorption spectra of 10 μg mL^{-1} Mefenamic acid and 6 μg mL^{-1} Piroxicam. HPSAM will be applied at the wavelengths indicated by the lines.

The appropriate wavelengths for the H-Point Standard Addition Method to be used were selected using the following criteria [21-22]:

i. At the two selected wavelengths, the signal from the sample should be linear with the analyte concentration. On the other hand, independent of fluctuations in the analyte concentration, the interference signal ought to remain constant.

ii. The total signals of the two species are equal to the analyte and interference analytical signals in the mixture.

iii. To achieve the best sensitivity, the chosen wavelengths increase steep slopes.

Mef and Pir can be viewed as analytes and interferences, respectively, in the current study. A number of wavelength pairs with the same Pir absorbance were examined in this instance and in light of the fact that the analyte concentration error decreases with increasing slope value, as shown in Table 1. After adding known analyte quantities to the mixture one after the other, the mixture's absorbance at the two chosen wavelengths was measured. Since it results in the largest higher slope for the Mef line and the largest difference in the slope values of the standard addition curves (0.0116 at 305.3 nm and 0.027 at 343.1 nm), 305.3 nm and 343.1 nm were the best pair. The HPSAM curves for Mef as the analyte and Pir as the interference at the two chosen wavelengths are displayed in Figure 3. At the intersection of the x-axis, the concentration of Mef was immediately ascertained.

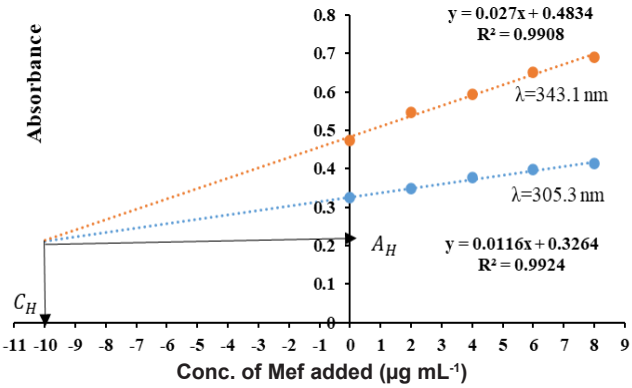

Figure 3. Plot the HPSAM curves for 10 μg mL^{-1} Mef and 6 μg mL^{-1} Pir at the chosen wavelengths (305.3 and 343.1 nm).

Applicability of the H-point standard addition technique

The suggested procedure's applicability to deter Mef and Pir has been clarified after using it on a number of synthesized samples and demonstrating that C_H (analyte concentration) is independent of interference concentration and, consequently, A_H (absorbance corresponding to interference concentration) is independent of analyte concentration. The findings from the HPSAM application are in good accordance with the quantities found in the spiked samples, as shown in Figure S1, S2, *Supplementary Materials*. The limit of detection was determined as LOD = $3S_{CH}$, where S_{CH} is the standard deviation of (n = 3) repeated measurements of zero analyte concentration. The calculated value was 0.007 μg mL^{-1} for Mef and Pir.

Table 1. The proper wavelength selection for mixture analysis.

Wavelength (nm)	Equations	R^2 Mef	Amount taken (μg mL^{-1})		Amount found (μg mL^{-1})	Absorbance (A_H)
			Pir	Mef	Pir	
305.3	$Y_{305.3}$=0.0116x+0.3264	0.9924	10.0	6.0	10.19	0.2081
343.1	$Y_{343.1}$=0.027x+0.4834	0.9908				
309.0	$Y_{309.0}$=0.0079x+0.3356	0.9095	10.0	6.0	10.56	0.2521
339.5	$Y_{339.5}$=0.0232x+0.4972	0.9872				
300.0	$Y_{300.0}$=0.0113x+0.3908	0.9615	10.0	6.0	11.16	0.2646
346.4	$Y_{346.4}$=0.0221x+0.5114	0.9977				

Calibration curve of Piroxicam

A calibration graph is necessary to calculate the interference concentration using the ordinate value of the H-point (A_H). Different solutions with constant Mef concentration (10 μg mL^{-1}) and varying Pir concentrations (0–10 μg mL^{-1}) were supplemented with specific Mef concentrations. In Figure S3, *Supplementary Materials* the absorbance at 305.3 and 343.1 nm was plotted versus the addition of Mef concentrations. Plotting the resulting A_H values against the Pir concentration, (0–10 μg mL^{-1}), yields a calibration curve that is used to determine the concentration of Pir interference Figure 4. A commendable recovery was attained for Mef and Pir in the mixture, as illustrated in Table S1, *Supplementary Materials*.

Accuracy

Using the recommended technique, a number of synthetic samples with varying concentration ratios of Pir and Mef acid were examined. Table S2, *Supplementary Materials* shows that, for the concentration ratio of mefenamic acid to piroxicam, which ranges from 2:4 to 10:6. The range of 2.0–10.0 μg mL^{-1} for both Mef and Pir, was revealed by the analysis of the various mixes using the suggested approach. As shown in Table S2, the accuracy of the results is good.

Reproducibility

The proposed method's repeatability was tested by determining the relative standard deviations of computed Mefenamic acid and Piroxicam concentrations. As shown in Table S3, *Supplementary Materials*, the suggested approach has good repeatability for the simultaneous determination of Mefenamic acid and Piroxicam.

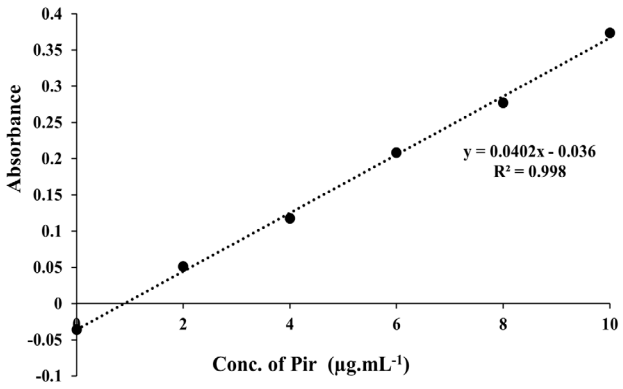

Figure 4. Calibration graph of Pir in the presence 10 μg mL^{-1} Mef.

Applications

Mef and Pir were successfully determined in pharmaceutical formulations using the H-point test method; the results were satisfactory and in line with the amounts indicated. Table 2 shows that no excipient influence was detected using the standard addition method.

Statistical analysis

Using ANOVA single factor, the proposed H-point technique was statistically evaluated for each species with three other methods (RP-HPLC [18], PLS [14], and ANN [19]). As shown in Table 3, the high value of the F statistic and the p value less than 0.05 indicate a significant difference between the averages of the four groups. The null hypothesis is thus rejected. The H-point technique exhibits the lowest variance, demonstrating the method's exceptional precision.

Table 2. Determination of Mef in the presence of Pir in pharmaceutical preparations.

Equation	R^2	Analyte Conc. (μg mL^{-1}) Mefenamic acid Ponamec syrup 50mg/5mL MVC, India				Interference Conc. (μg mL^{-1}) Piroxicam Feldene ampoule 20mg/mL Pfizer-France			
		Taken	Found	Rec %	SD	Taken	Found	Rec %	SD
$Y_{305.3}$=0.0052x+0.207	0.9657	5.0	4.75	96.4	0.09	6.0	5.43	92.0	0.16
$Y_{343.1}$=0.0132x+0.244	0.9963								
$Y_{305.3}$=0.0047x+0.216	0.9601		4.78				5.71		
$Y_{343.1}$=0.0129x+0.255	0.9889								
$Y_{305.3}$=0.0049x+0.206	0.9502		4.93				5.43		
$Y_{343.1}$=0.0126x+0.245	0.9873								
$Y_{305.3}$=0.0076x+0.243	0.9953	7.0	7.06	102	0.19	6.0	5.60	92.8	0.05
$Y_{343.1}$=0.0151x+0.296	0.9949								
$Y_{305.3}$=0.0061x+0.234	0.9800		7.36				5.61		
$Y_{343.1}$=0.0133x+0.288	0.9955								
$Y_{305.3}$=0.0072x+0.236	0.9254		7.00				5.51		
$Y_{343.1}$=0.0142x+0.286	0.9864								

Table 3. Statistical comparison between the results of the suggested method and three other methods (RP-HPLC, PLS, and ANN) using one-way ANOVA.

SUMMARY STATISTIC OF MEFENAMIC ACID					
Groups	**Count**		**Sum**	**Average**	**Variance**
RP-HPLC	3		35.6106	11.8702	0.309183
PLS	3		14.35886	4.786287	0.006926
ANN	3		5.978	1.992667	0.001827
H-point	3		12.1	4.033333	0.001733
ANOVA					
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	166.2747	3	55.42489	693.5273	5.29E-10	4.066181
Within Groups	0.639339	8	0.079917			
SUMMARY STATISTIC OF PIROXICAM					
Groups	**Count**		**Sum**	**Average**	**Variance**
RP-HPLC	3		34.6066	11.53553	0.31934
PLS	3		14.88684	4.96228	0.041986
ANN	3		5.93	1.976667	0.001228
H-point	3		7.12	2.373333	0.000133
ANOVA					
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	175.7245	3	58.57484	646.0092	7.02E-10	4.066181
Within Groups	0.725375	8	0.090672			

Conclusion

The H-Point test method was effectively used to identify piroxicam and mefenamic acid in both their pharmaceutical and binary combination forms. The suggested approach is straightforward, accurate, sensitive, and might be applied to routine analysis with basic tools or technology. This approach requires more time and computations but no additional processing.

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