Technical Paper

Simultaneous Determination of Salicylamide and Paracetamol by Spectrophotometric H-Point Standard Addition Method and Partial Least Squares Regression

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Abstract

Simultaneous spectrophotometric determination of salicylamide and paracetamol by H-point standard addition method (HPSAM) and partial least squares (PLS) calibration is described. The results showed that simultaneous determinations could be performed with the ratio 0.2:5–20:1 for salicylamide – paracetamol. A partial least – squares multivariate calibration method for the analysis of binary mixtures of paracetamol and salicylamide was also developed. The total relative standard error for applying the PLS method to 10 synthetic samples in the concentration range 0–60 μg mL^{-1} salicylamide and 0–30 μg mL^{-1} paracetamol was 5.1%. Both the proposed methods (PLS and HPSAM) were successfully applied to the determination of salicylamide and paracetamol in pharmaceutical preparations.

Key words: Salicylamide, Paracetamol, Simultaneous determination, HPSAM, PLS

Introduction

Acetaminophen (*N*-acetyl-*p*-aminophenol; paracetamol) and salicylamide (*o*-hydroxybenzamide) have been widely used as analgesic and antipyretic drugs. They are frequently prescribed in admixture with each other or with other related drugs.

Several methods have been reported for simultaneous determination of salicylamide and paracetamol. These include HPLC,[1] spectrofluorimetric,[2] electrochemical,[3] and spectrophotometric[4] methods.

The H-point standard addition method[5] (HPSAM) permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. This method was based on the principle of dual wavelength spectrophotometry and the standard addition method. The greatest advantage of HPSAM is that, it can remove the errors resulting from the presence of an interfering and blank reagent. Although HPSAM could remove the error resulting from the sample matrix, it cannot remove the constant error resulting from other components in the system. The requirements for the application of the method is that if necessary to work only at two wavelengths where the analytical signal due to the one of the species is constant and for another one to be as different as possible. By plotting the analytical signal versus the added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H), where $-C_H$ is the unknown analyte concentration and A_H the analytical signal due to the interfering species.

In recent years, multivariate calibration methods have an increasing importance in multicomeponent analysis, especially those using the (PLS) method with decomposition into latent variables. Interest in UV-VIS spectrophotometric methods has increased and been renewed through the use of signal processing and multivariate techniques[6] such as partial least squares (PLS) regression[7,8] and artificial neural networks.[9] These tools can allow simultaneous spectrophotometric determination of several elements and drugs as well as improve the data handling process of complex chemical systems.

In this work HPSAM and PLS were employed for the resolution of binary mixtures of paracetamol and salicylamide. The suggested methods were successfully applied to the determination of these analytes in pharmaceuticals.

Results and discussion

Figure 1 shows the absorption spectra of salicylamide and acetaminophen. As can be seen, the spectra of both the compounds show a strong overlapping hindering the resolution of their mixture by conventional spectrophotometry. However, the system is suitable for the simultaneous determination salicylamide and paracetamol using HPSAM.

In order to find the optimum pH for the determination of salicylamide and paracetamol by spectrophotometric method, the influence of pH in the range 2–9 on their spectra was investigated. The results showed that pH in the range 2–9 had no effect on the determina-

Table 1. Characteristics of calibration graphs for the determination of salicylamide and paracetamol by the proposed method.

Analyte	Slope	Intercept	Correlation Coefficient	Linear range /μg mL^{-1}	Limit of Detection /μg mL^{-1}
Salicylamide	0.0267	0.02	0.999	0.20 – 60	0.09
Paracetamol	0.0659	0.174	0.9987	0.50 – 30	0.31

tion of salicylamide and paracetamol. Therefore routine works were performed at pH 6.

Individual calibrations

To verify the governing Beer's law, calibration graphs were prepared for the determination of salicylamide and paracetamol at pH 6. Characteristics of the calibration graphs are given in Table 1. The Limit of detection was defined as $C_L = 3S_B/m$, where C_L, S_B and m are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively.[10] The appropriate correlation coefficients obtained indicate that the interaction between the two binary systems either does not exist or at least does not affect the linear correlation prevailing between absorbance and concentration of each drug. Thus, chemometric methods based on factor analysis such as PLS seem to be suitable for use in this system.

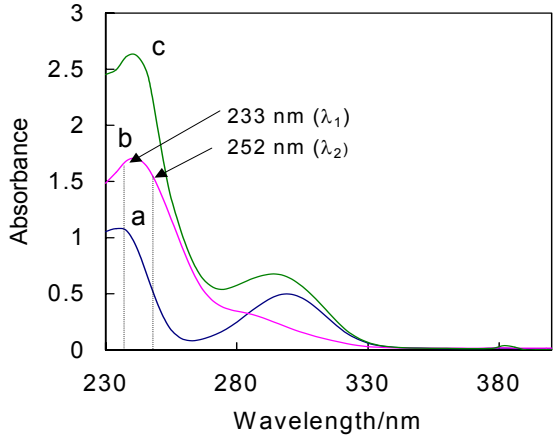

Figure 1. Absorption spectra of 20 μg mL^{-1} salicylamide, (a) 20 μg mL^{-1} paracetamol (b) and their mixture (c) at pH 6.

Requirements for applying HPSAM

Consider an unknown sample containing an analyte X and an interferent Y. The determination of concentration of X by HPSAM under these conditions requires the selection of two wavelengths λ_1 and λ_2 at which the interfering species, Y, should have the same absorbance. Then known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two wavelengths and expressed by equations (1) and (2), where $A_{(\lambda1)}$ and $A_{(\lambda2)}$ are the analytical signals measured at λ_1 and λ_2, respectively; b_0 and A_0 ($b_0 \neq A_0$) are the original analytical signal of X at λ_1 and λ_2, respectively; b and A' are the analytical signals of Y at λ_1 and λ_2, respectively; $M_{\lambda1}$ and $M_{\lambda2}$ are the slopes of the standard addition calibration lines at λ_1 and λ_2, respectively; C_i is the added X concentration. The two straight lines obtained intersect at the so-called H point ($-C_H$, A_H), (Figure 2, equations (1) and (2)).

At the H-point, since $A_{(\lambda1)} = A_{(\lambda2)}$, $C_i = -C_H$ from equations (1) and (2) follow equations (3) and (4).

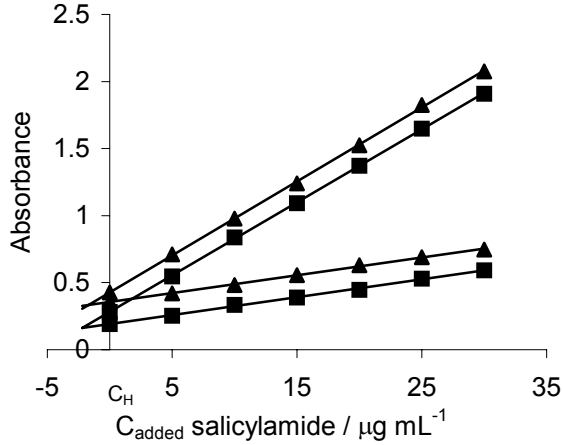

Figure 2. Plots of H-point standard addition method for fixed salicylamide (2 μg mL^{-1}) and (■) 2 and (▲) 5 μg mL^{-1} of paracetamol.

From Equation (4), the following conclusions can be obtained: (i) If the Y component is the known interferent and the analytical signal corresponding to Y, b (at λ_1) and A' (at λ_2) do not change with the additions of an analyte, X, that is, b = A' = constant, and thus see equations (5)–(8).

$$A_{(\lambda1)} = b_0 + b + M_{\lambda1} C_i, \quad (1)$$

$$A_{(\lambda2)} = A_0 + A' + M_{\lambda2} C_i, \quad (2)$$

$$b_0 + b + M_{\lambda1}(-C_H) = A_0 + A' + M_{\lambda2}(-C_H), \quad (3)$$

$$-C_H = [(A_0 - b_0) + (A' - b)] / (M_{\lambda1} - M_{\lambda2}). \quad (4)$$

$$C_X = (A_0 - b_0)/M_{\lambda1} - M_{\lambda2}) = b_0/M_{\lambda1} = A_0/M_{\lambda2}, \quad (5)$$

If $C_H = -C_x$ then,

$$-C_H = (A_0 - b_0) / (M_{\lambda1} - M_{\lambda2}) = b_0/ M_{\lambda1} = A_0/ + M_{\lambda2} \quad (6)$$

If the value of $-C_H$ is included in Equation (1), then

$$A_H = b_0 + b + M_{\lambda1} (-C_H) \quad (7)$$

$B_0 = -M_{\lambda1} C_H$ (Equation (4)), then

$$A_H = b \quad (8)$$

And similarly

$$A_H = A'.$$

Table 2. Results of several experiments for the analysis of salicylamide-acetaminophen mixtures at different concentration ratios by HPSAM.

A– C Equation	R	Present in sample / μg mL^{-1}		Found / μg mL^{-1}	
		Salicylamide	Paracetamol	Salicylamide	Paracetamol
$A_{233} = 0.3425 + 0.054C_i$	0.999	0.20	5.00	0.20	4.96
$A_{252} = 0.3345 + 0.014C_i$	0.996				
$A_{233} = 0.3088 + 0.0536C_i$	0.999	3.00	2.00	2.98	2.06
$A_{252} = 0.1878 + 0.0130C_i$	0.999				
$A_{233} = 0.439 + 0.058C_i$	0.998	5.00	2.00	4.93	2.10
$A_{252} = 0.213 + 0.01216C_i$	0999				
$A_{233} = 0.272 + 0.0545C_i$	0.999	2.00	2.00	1.99	2.02
$A_{252} = 0.190 + 0.0133C_i$	0.997				
$A_{233} = 0.4283 + 0.055C_i$	0.999	2.00	5.00	1.88	4.75
$A_{252} = 0.3494 + 0.013C_i$	0.998				
$A_{233} = 0.3987 + 0.05546C_i$	0.999	1.00	5.00	0.93	5.10
$A_{252} = 0.3591 + 0.01286C_i$	0.996				
$A_{233} = 0.2654 + 0.054C_i$	0.999	1.00	3.00	1.04	2.99
$A_{252} = 0.2228 + 0.0131C_i$	0.999				
$A_{233} = 0.987 + 0.051C_i$	0.999	17.00	1.00	17.50	1.00
$A_{252} = 0.304 + 0.012C_i$	0.998				
$A_{233} = 1.199 + 0.05C_i$	0.999	20.00	1.00	20.57	1.00
$A_{252} = 0.372 + 0.00979C_i$	0.998				

Hence, A_H value is only related to the signal of the interference Y at the two selected wavelengths and C_H is independent from the concentration of interference. Figure 2 shows the effect of change in concentration of paracetamol on the position of the H-point.

(ii) If component Y is the unknown interferent, Equation (4) is tenable as long as the Y analytical signals (b at λ_1 and A' at λ_2) remain equal with the addition of analyte X.

According to the above discussion at H point, C_H is independent from the concentration of interferent and so, A_H is also independent from the analyte concentration.

For selection of appropriate wavelengths for applying HPSAM, the following principles were followed. At two selected wavelengths, the analyte signals must be linear with the concentrations, the interferent signals must remain equal, even if the analytical concentrations are changed, and the analytical signals of the mixture composed from the analyte and the interferent should be equal to the sum of the individual signals of the two compounds. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 must be as large as possible in order to get good accuracy.

In this special system, analyte is salicylamide and paracetamol is as interferent. Several wavelength pairs were examined and the wavelength pair of 233 and 252 nm was selected. Under optimum conditions, determination of salicylamide and paracetamol was carried out using HPSAM. The concentration of interferent was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H- point (A_H).

Several synthetic mixtures with different concentration ratios of salicylamide and paracetamol were analyzed by the proposed method. The results are given in Table 2.

Repeatability of the HPSAM

Simultaneous determination of salicylamide and paracetamol at pH 6.0 were made using HPSAM. To check the repeatability of the method five replicate experiments of the salicylamide and paracetamol were done (Table 3). Then the concentration of interferent was calculated in each test solution by calibration method using standard solutions and the ordinate value of H- point (A_H). The relative standard deviations (RSD) for five replicate measurements of the mixture of 2.0 μg mL^{-1} each of paracetamol and salicylamide were1.5 and 2.48%, respectively.

Partial least squares (PLS) regression

The calibration procedure consisted of a complete experimental design with six concentration levels for both paracetamol and salicylamide.

Each solution was prepared to contain combinations of the concentration levels (0.0–60 μg mL^{-1} of salicylamide and 0.0–30 μg mL^{-1} of paracetamol). A set of 33 mixtures of paracetamol and salicylamide are shown in Table 4.

Table 3. Results for five replicate for the analysis of paracetamol – salicylamide mixtures by HPSAM.

A– C Equation	R	Present in sample /μg mL^{-1}		Found / μg mL^{-1}	
		Salicylamide	Paracetamol	Salicylamide	Paracetamol
A_{233} 0.297 + 0.053C_i	0.999	2.00	2.00	1.94	2.06
A_{252}= 0.220+ 0.0133C_i	0.999				
A_{233} = 0.272 + 0.0545C_i	0.999	2.00	2.00	1.99	2.02
A_{252}= 0.190 + 0.0133C_i	0.998				
A_{233}= 0.241 + 0. 055C_i	0.999	2.00	2.00	2.02	2.04
A_{252}= 0.156+ 0.013C_i	0.996				
A_{233}= 0.278 + 0. 053C_i	0.999	2.00	2.00	2.00	1.93
A_{252}= 0.195+ 0.0118C_i	0.996				
A_{233}= 0.275 + 0.0557C_i	0.999	2.00	2.00	2.00	2.00
A_{252}= 0.189+ 0.0127C_i	0.999				
Mean				2.01	1.99
Standard deviation				0.05	0.03
R.S.D (%)				2.48	1.50

Table 4. Values of the paracetamol and salicylamide concentrations used as calibration and prediction solutions in μg mL^{-1}.

Calibration set		Prediction set	
Paracetamol	Salicylamide	Salicylamide	Paracetamol
0	12	36	0
0	24	12	6
0	48	60	6
0	60	48	12
6	0	32	18
6	12	60	18
6	36	0	24
6	48	36	24
12	0	12	30
12	12	48	30
12	24	–	–
12	36	–	–
12	60	–	–
18	0	–	–
18	12	–	–
18	36	–	–
18	48	–	–
24	12	–	–
24	24	–	–
24	48	–	–
24	60	–	–
30	0	–	–
30	24	–	–

Twenty-three of these solutions were used as a calibration set for PLS model development. Another 10 calibration mixtures, not included in the previous set were employed as an independent test set called the prediction set. To select the number of factors in the PLS algorithm, the cross- validation method, employed was to eliminate only one sample at a time[11]. The prediction error was calculated for each component for the prediction set, which are the samples not participating in the construction of the model. The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the prediction error sum of squares (PRESS) for the first variable, which built the PLS modeling in the calibration step, then, another latent variable was added for the model building and the PRESS was calculated again. This process was repeated for one to 10 latent variables, which were used in the PLS modeling.

The predicted concentrations of the compounds in each sample were compared with the already known concentration and the prediction error sum of squares (PRESS) was calculated by each number of factors. Figure 3 shows a plot of PRESS against the number of factors for each individual component. The F-statistical test can be used to determine the significance of PRESS values greater than the minimum. The optimal number of factors for paracetamel and salicylamide was obtained too. The results obtained are given in Table 5. The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E) of the prediction concentration (Equation (9))[12,13] where N is the number of samples, Cj the concentration of the component in the jth mixture and $\hat{C}j$ the estimated concentration. The total prediction error of N samples is calculated as follows in Equation (10), where C_{ij} is the concentration of the ith component in the jth sample and $\hat{C}_{ij}$ its estimation. Table 5 also shows reasonable single and total relative errors for such a system.

Table 5. Composition of prediction set, their predictions by PLS model and statistical parameters for the system.

Composition (μg mL^{-1})		Prediction (μg mL^{-1})		Recovery (%)	
Salicylamide	Paracetamel	Salicylamide	Paracetamal	Salicylamide	Paracetamal
36.00	0.00	36.00	0.09	100.0	–
12.00	6.00	12.50	6.71	104.1	111.7
60.00	6.00	60.97	6.43	101.6	107.1
48.00	12.00	48.00	12.55	100.0	104.6
32.00	18.00	35.42	19.60	110.7	108.9
60.00	18.00	60.56	18.69	100.9	103.8
0.00	24.00	0.48	25.46	–	106.0
36.00	24.00	36.27	25.08	100.7	104.5
12.00	30.00	11.74	34.86	97.8	116.2
48.00	30.00	45.46	31.79	94.7	105.9
Mean recovery				101.1	108.6
R.S.E.(%) single				3.57	8.7

Table 6. Simultaneous determination of salicylamide and paracetamol in pharmaceutical preparations by HPSAM and PLS method.

Sample[a]	Nominal value / mg		Found[b] /mg	
	Salicylamide	Paracetamol	Salicylamide	Paracetamol
Yendol granular packet	500	200	510 ± 5[c] 493 ± 6[d]	207 ± 3[c] 201 ± 4[d]
Pridio capsules	100	300	104 ± 2[c] 96 ± 4[d]	299 ± 6[c] 299 ± 3[d]
Rinomicinc activated tablets	150	150	151 ± 5[c] 146 ± 6[d]	155 ± 7[c] 157 ± 5[d]
Rinomicinc pellets	50	50	50 ± 3[c] 49 ± 4[d]	50 ± 3[c] 49 ± 5[d]

[a] ***Composition of samples***: Yendol: Salicylamide, 500 mg; paracetamol. 200 mg; chlorpheniramine maleate, 3 mg; caffeine, 39 mg; saccharin, 10 mg; saccharose. 6.56 g. pridio: salicylamide, 100 mg; paracetamol, 300mg; caffeine, 25 mg. chlorpheniramine maleate, 2mg. Rinomicine activated: paracetamol, 150 mg; chlorpheniramine maleate, 4 mg; caffeine, 30 mg; Salicylamide, 150 mg; phenlephrine hydrochloride. 10 mg; Rinomicine: paracetamol, 50mg; chlorpheniramine maleate, 4 mg; Salicylamide, 50 mg; phenlephrine hydrochloride. 20 mg. [b] Mean ± S. D. (n=3). [c] By HPSAM. [d] By PLS method.

$$\text{R.S.E.}(\%)=\left(\sum_{i=1}^{M}(\hat{C}-C_j)^2/\sum_{j=1}^{N}(C_j)^2\right)^{0.5}\times 100 \quad (9)$$

$$\text{R.S.E.}_t(\%)=\left(\sum_{i=1}^{M}\sum_{j=1}^{N}(\hat{C}_{ij}-C_{ij})^2/\sum_{i=1}^{M}\sum_{j=1}^{N}(C_{ij})^2\right)^{0.5}\times 100 \quad (10)$$

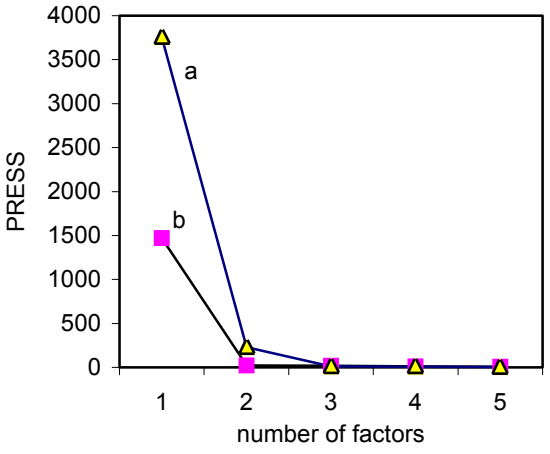

Figure 3. Plot of PRESS against the number of factors for (a) paracetamol and (b) salicylamide.

Application

To evaluate the analytical applicability of both the proposed methods, (PLS and HPSAM) they were applied to the simultaneous determination of salicylamide and paracetamol in pharmaceutical preparations containing both compounds. The results are given in Table 6. The good agreement between the results with the composition values indicated by the suppliers indicates the successful applicability of the proposed methods for simultaneous determination of salicylamide and paracetamol in pharmaceutical preparations.

Conclusion

The above results show that HPSAM and PLS regression allow rapid, accurate and simple resolution of paracetamol and salicylamide mixtures.

The HPSAM can be used in the complex samples with matrix effects because standard addition method

has capability of removing these effects. But partial least squares regression cannot be used in these cases. On the other hand the PLS method was more rapid than HPSAM. Therefore in the mixtures with matrix effects HPSAM is preferred but in the mixtures without these effect PLS is better than HPSAM because of rapidity.

Experimental

Reagents

Triply distilled water and analytical–reagent grade chemicals were used. A 1000 μg mL^{-1} salicylamide (Aldrich) solution was prepared in 5% ethanol (Merck)/ water (v/v); this solution was stable for at least two weeks. Working solutions were prepared daily by diluting the standard solution with water. Standard paracetamol solution, 1000 μg mL^{-1} of 4-acetamidophenol (Merck) in water was prepared daily. Working solutions were prepared by diluting the standard solution with water.

Apparatus

A Pharmacia model LKB3 UV-Visible Ultraspect3 single beam spectrophotometer with 1-cm quartz cells, connected to a Pentium II computer was used for absorbance measurements. All spectral measurements were performed using the blank solution as a reference. Measurements of pH were made with a Jenway C15 pH- meter using a combined glass electrode. The computations were made with a Pentium4 computer. All programs in the computing process were written in MATLAB for windows.

Procedure

A 1 mL of pH 6 buffer solution and appropriate volumes of salicylamide and acetaminophen solutions were added to a 10 mL volumetric flask and made up to the mark with water and mixed well. Simultaneous determination of salicylamide and acetaminophen with HPSAM was performed by measuring the absorbance of the solution at 233 and 252 nm for each sample. Synthetic samples containing different concentration ratios of salicylamide and acetaminophen were prepared and standard addition of salicylamide were made. The concentration range of salicylamide and acetaminophen for the construction of HPSAM calibration graphs were 0.2–30 and 0.5–30 μg mL^{-1}, respectively.

Simultaneous determination of salicylamide and acetaminophen with PLS method was performed by recording the absorbance spectra for each solution from 230 to 330 nm. The concentration range of salicylamide and paracetamol in the PLS method in the same conditions was 0.0–60 μg mL^{-1} and 0.0–30 μg mL^{-1}, respectively.

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Povzetek

Opisano je sočasno spektrofotometrično določevanje salicilamida in paracetamola z metodo standardnega dodatka pri dveh valovnih dolžinah (HPSAM) in umeritvijo na osnovi parcialne regresijske analize (PLS). Rezultati so pokazali, da je sočasno določevanje obeh spojin možno v območju razmerij salicilamid:paracetamol od 0,2:5 do 20:1. Skupna relativna standardna napaka pri analizi 10 sintetičnih vzorcev binarnih zmesi s koncentracijami salicilamida 0 – 60 μg mL^{-1} in paracetamola 0 – 30 μg mL^{-1} z metodo PLS je bila 5,1 %. Obe metodi (PLS in HPSAM) sta bili uspešno uporabljeni za določevanje salicilamida in paracetamola v farmacevtskih pripravkih.