Simultaneous Determination of Acetyl Salicylic Acid and Acetaminophen in Acetaminophen-Caffeine-Aspirin (ACA) Tablets by FT-IR/ATR Spectrometry with Multivariate Calibration Data Treatment

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A simultaneous method based on Fourier transform infrared (FTIR) was developed for the determination of acetyl salicylic acid (aspirin) (ASA) and acetaminophen coupled with an attenuated total reflectance (ATR) accessory. The method was based on dissolving the drug powder in ethanol followed by FTIR spectroscopy and prediction of the active components using partial least squares (PLS) regression analysis. Although the drug mixture showed some regions of spectra overlapping, but they were simultaneously determined with high accuracy, without interference from tablet excipients. A comparison is represented with related multivariate method of principal component regression (PCR) analysis, which is shown to yield less reliable results. In the analysis of real and synthetic samples, precise and accurate values were obtained by PLS-FTIR with less than 3% standard errors. Finally, the results were compared with standard method (USP) and good agreements were obtained by PLS-1.

Key Words: Pharmaceutical analysis, Acetyl salycylic acid and acetaminophen, Quantitative determination, FT-IR/ATR spectroscopy, Multivariate calibration.

INTRODUCTION

Acetaminophen[N-(4-hydroxyphenyl)acetamide, 4′-[hydroxyacetanilide] and acetyl salicylic acid [2-(acetyloxy)benzoic acid, 2-carboxyphenylester] are active principle analgesic drugs, which occur in combination in a number of pharmaceutical formulations, including tablets. Therefore, the determination of the two drugs is a frequent analytical problem in quality control of the pharmaceutical industry. It is reported that FT-IR spectroscopy has better selectivity than UV/Vis spectroscopy and that it is an excellent tool for the determination of drugs in mixtures[1,2].

FT-IR spectroscopy is reported for the simultaneous on-line quality control of acetyl salicylic acid and caffeine without requiring a previous separation[3] and can be used as a detection method in the analysis of mixtures, such as, acetaminophen, aspirin and caffeine by HPLC[4], TLC[5], UV/Vis[6] and stopped flow FT-IR[7].

The most commonly used multivariate techniques in conjunction with FT-IR spectroscopy are PLS and PCR, which have the ability to model non-linear responses[8,9]. In this work simultaneous determination of acetaminophen and acetyl salicylic acid by PLS-FTIR/ATR and PCR was used.

PLS is faster than PCR for modeling calibration sets, and can also model variations in the concentration matrix. Whereas PCR can only concentrate on the spectra matrix, PLS is widely employed in multicomponent quantitative analysis for several spectra data, such as IR and UV/Vis. This procedure is based on the regression of chemical concentrations on latent variable obtained from the spectral data. The main advantage of PLS calibration procedure is that it can model a system even in the presence of interfering signal, provided that they are included in the calibration steps. There are two main PLS algorithms named PLS-1 and PLS-2[10].

In PLS-1, a calibration model is built for each component, which means that for a two-component sample (as in this study), two different models should be built. PLS-2 is better suited for multicomponent analysis, because it can model several components simultaneously.

The main objective of this study was to develop a PLS-FTIR procedure for fast and accurate simultaneous determination of binary mixture in commercial pharmaceutical formulation and also providing a direct FT-IR determination by using a simple matrix calibration.

EXPERIMENTAL

Aspirin and acetaminophen were purchased from Darou Pakhsh-Tolid Darou and Alborz Darou (Iran). All other chemicals and solvents were of Merck reagent grade.

A Bomem 100 FT-IR spectrometer equipped with a spacer ZnSe ATR flat crystal accessory was used with a glowbar source and a DTGS detector. The liquid chromatography (HPLC), kenaver (Australia), equipped with a 275 nm detector (K-2500) and a 4.6 mm × 15 cm column, with 5 m packing L1 was used, and the temperature was maintained at 45 ± 1°C. The flow rate was about 2 mL per min, as suggested in USP[11]. The mobile phase was a mixture of water, methanol and glacial acetic acid (69 : 28 : 3) and the solvent was a mixture of methanol and glacial acetic acid (95 : 5).

Procedure: Accurate amounts of acetyl salicylic acid (0–4 g) and acetaminophen (0–4 g) were placed in a 100 mL calibration flask and 20 mL ethanol was added. The flask was shaken for 10 min and then diluted to volume with ethanol. Ten units of ACA tablets were ground and mixed thoroughly and appropriate amounts of sample were taken for analysis by both FT-IR and reference HPLC analysis[11].

PLS and PCR FT-IR determination of ASA and acetaminophen: Accurate concentrations of ASA and acetaminophen (1–4 g)/100 mL were transferred to the measurement (ATR), respectively, and the FT-IR data were obtained. Ethanol was employed to establish the background spectrum. Synthetic mixtures

of the two compounds were used to obtain a total of 16 concentrations of which 9 were employed for establishing the calibration matrix and the remaining concentrations for testing the calibration model.

Multivariate method of data analysis: Multivariate data analysis was performed using Matlab. The PLS-toolbox was used with Matlab and Microsoft Excel (version 5.0).

RESULTS AND DISCUSSION

The choice of solvent is very important in IR spectroscopy determinations because some analgesic drugs are usually administered in high doses and the selected solvent must allow the drug to dissolve in fairly high concentration. Additionally, the solvents used must be transparent in the MID-IR range.

In this study, ethanol was used as a solvent because caffeine and other excipients are not dissolved in the solvent.

FT-IR spectra of standard: Fig. 1 shows the FT-IR spectra of acetyl salicylic acid and acetaminophen obtained in ethanol which indicates strong absorptions at 1200–1800 wave numbers with an intensive peak at 1500 wave number for the acetaminophen. However, it can be observed that the spectra of the two drugs show some regions of overlapping, and that the peaks cannot be resolved by traditional procedures using univariate calibrations. Therefore, for this reason the multivariate data treatment was carried out.

PLS and PCR calibration: The PLS and PCR methods require careful

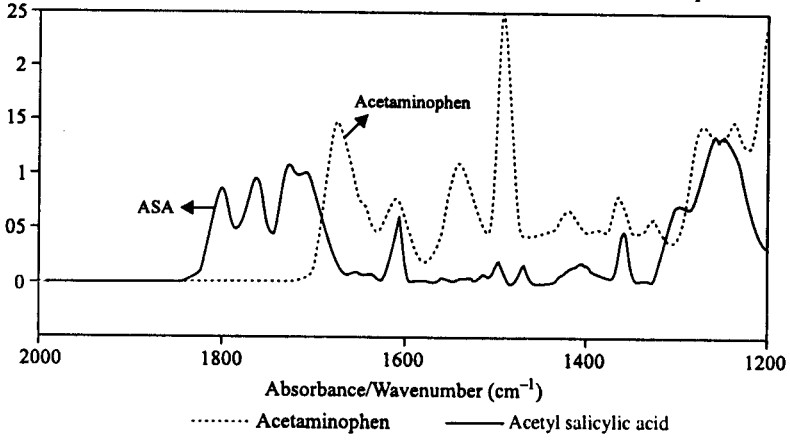


Fig. 1. FT-IR spectra of acetyl salicylic acid 2 g/100 mL and acetaminophen 2 g/100 mL

experimental design of standard composition of the calibration sets in order to provide the best predictions. Therefore, experimental design was generated by using a two-level factorial design for the two compounds. Thus, nine standard mixtures randomly were selected to construct the calibration. The results are shown in Table-1.

TABLE-1
COMPOSITION OF THE CALIBRATION MATRIX

Sample	Acetyl salicylic acid *	Acetaminophen*
1	0	0
2	0	2
3	0	4
4	2	0
5	2	2
6	2	4
7	4	0
8	4	2
9	4	4

g/100 mL*

PLS and PCR treatment of FT-IR data: From the FT-IR spectra of acetyl salicylic acid and acetaminophen (Fig. 1), a total of 416 data were extracted between absorptions at 1200–1800 wave numbers in order to give least amounts of mean squares error of prediction (MSEP). It was found that absorptions at 1470–1780 wavenumbers gave the least MSEP which reduced the above data to that of 160. Hence, in order to model a system for achieving an optimum factor in PLS and PCR algorithms, a CROSS validation method was used for the calibration sets. Thus, the plots obtained for the acetyl salicylic acid and acetaminophen are shown in Fig. 2a–b, respectively, and the results obtained are shown in Table-2. It should be noted that the values presented in Table-2 for PLS-2 and PCR are for both acetyl salicylic acid and acetaminophen as same values were obtained for both drugs. As it can be observed from Fig. 2 and Table-2, this stage was repeated for test sets (external validation). So, the predicted concentrations of compounds in the sample left were compared with the actual concentrations in the reference sample. The prediction error sum of squares (PRESS) were calculated for each method from

$$\text{PRESS} = \sum_{i=1}^{n} (C_i - C_i')^2$$

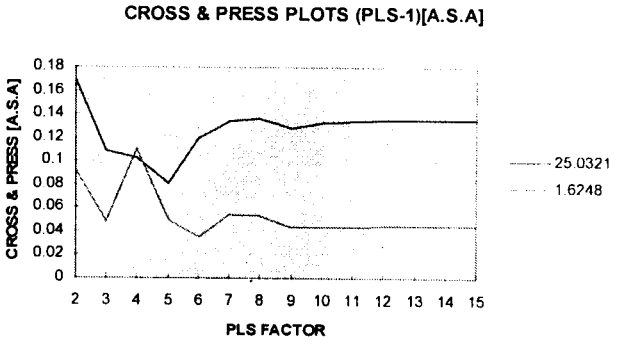
where n is the number of compounds, C_i is the actual concentration of the analyst in samples C_i and C_i' is the predicted concentration of the analyst in the samples.

TABLE-2
VALUES OF CROSS, PRESS AND MSEP *vs.* NUMBER OF FACTORS
(PLS-1, PLS-2, PCR)

Method	No. Factor	CROSS	No. Factor	MSEP	PRESS
PLS-1*	5	0.0805	6	0.0022	0.0352
PLS-1†	4	0.0537	3	0.0038	0.0488
PLS-2	4	0.1477	3	0.0037	0.1184
PCR	4	0.1233	4	0.1214	0.0038

*Aspirin (acetyl salicylic acid); †Acetaminophen,

(a)



(b)

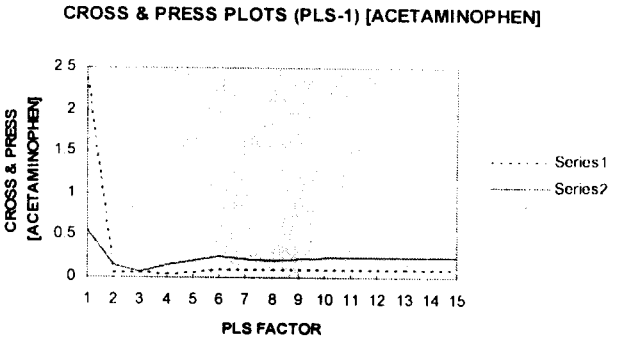


Fig. 2. Value of CROSS and PRESS *vs.* the number of factors (PLS-1): (a) for acetyl salicylic acid and (b) for acetaminophen, respectively

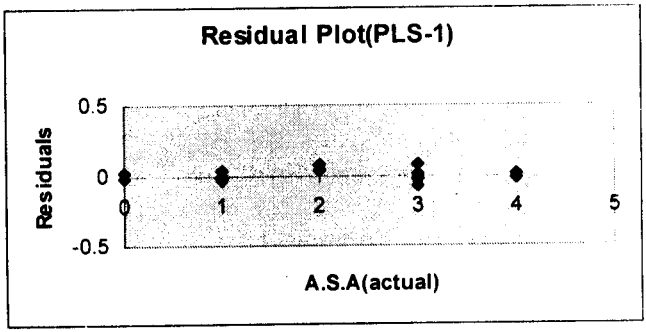
For finding the anomalous compounds, residual concentrations *vs.* actual concentrations were plotted and, as a result, no outliers were observed (see Fig. 3). For calibration sets, the amounts of correlation coefficient and root mean square error of prediction (RMSEP) were determined for all the methods (PLS-1, PLS-2 and PCR), which are shown in Table-3. From Table-3, it can be observed that the best results were obtained for PLS-1. The predicted and the real concentrations for the acetyl salicylic acid and acetaminophen (PLS-1 model) are shown in Fig. 4a–b, respectively, which shows that the results obtained are in good agreement.

TABLE-3
STATISTICAL PARAMETERS (PLS-1, PLS-2 AND PCR CALIBRATION)

Method	R^2	RMSEP
PLS-1*	0.9999	0.0135
PLS-1†	0.9992	0.0428
PLS-2*	0.9990	0.0534
PLS-2†	0.9992	0.0482
PCR*	0.9994	0.0403
PCR†	0.9993	0.0429

*Aspirin (acetyl salicylic acid); †Acetaminophen

(a)



(b)

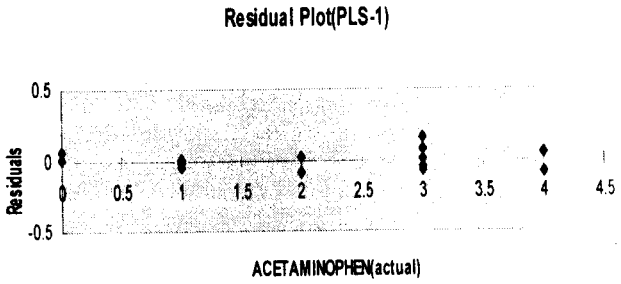
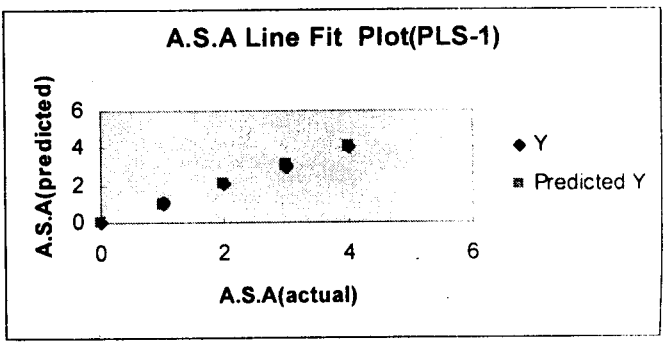


Fig. 3. Values of residuals *vs.* actual sample (outlier detection): (a) for acetyl salicylic acid and (b) for acetaminophen, respectively

(a)



(b)

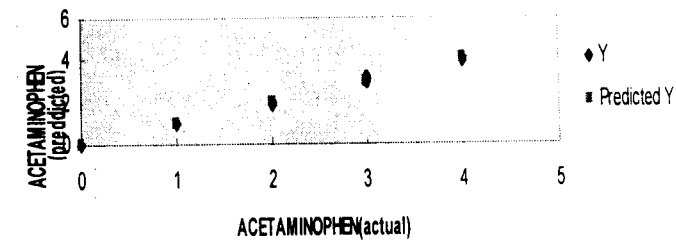


Fig. 4. Values of predicted sample *vs.* actual sample (PLS-1): (a) for acetyl salicylic acid and (b) for acetaminophen, respectively

Analysis of commercial tablets: Commercial acetyl salicylic acid and acetaminophen tablets were analysed by PLS-1/FTIR and USP standard method. The results obtained are shown in Table-4. As can be observed, the difference between the two methods is not significant.

TABLE-4
RESULTS SHOWING PLS-1 ANALYSIS AND STANDARD METHOD FOR DETERMINATION OF ACETYL SALICYLIC ACID AND ACETAMINOPHEN

Method	Acetyl salicylic acid	Acetaminophen
Content	325.00	162.50
PLS-1*	339.00	169.00
PLS-1†	341.00	171.00
USP*	338.54	168.12
USP†	342.07	173.08

*Tolid Darou; †Alborz Darou

Conclusions

This study has demonstrated a great potential for using PLS-1 for the FT-IR control analysis of pharmaceutical compounds. Although the drug mixtures exhibited some regions of spectra overlapping, but it was possible simultaneously to determine a good correlation between the reference and the predicted values for acetyl salicylic acid and acetaminophen with less than 3% standard errors. The results obtained by PCR showed to be less reliable methods compared to PLS. It was found that PLS method is simple, fast and inexpensive for multicomponent analysis and is not time consuming for extraction stages and large volumes of solvent, unlike USP method[11] which is not required.

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