Research Paper

Development and Validation of an Alternate Stability-indicating UV Spectrophotometric Analytical Method for Aspirin in Tablets

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Aspirin is a drug that has been used for widely for more than a century. Numerous stability-indicating analytical methods have been reported over the years. A new stability indicating method was developed for the accurate quantitation of aspirin in tablets using the partial least squares calibration algorithm. This method is specific for the determination of aspirin and its main degradation product; salicylic acid in tablets using ultraviolet spectrophotometry. The calibration matrix needs only 13 binary solutions and the method showed good linearity from 30 to 70 µg/ml for aspirin and 0 to 40 µg/ml for salicylic acid. Moreover, a full validation according to the International Council of Harmonization guidelines was carried out covering precision, recovery, sensitivity, sample stability and tolerance. The method was applied to developed and commercial aspirin tablets. The proposed method represents a reliable choice to be used as a routine method for quality control of newly manufactured products.

Key words: Aspirin, stability-indicating method, partial least squares, UV spectrophotometry, validation

Aspirin (ASP) also known as acetylsalicylic acid is a well-established drug used worldwide as an and analgesic, antipyretic and antiinflammatory drug[1-3]. ASP along with its main and active degradation product salicylic acid (SA) has been studied for decades. Basically, once ASP is ingested, it is immediately hydrolysed to SA, which is responsible for the pharmacological activity (fig. 1)[4]. This apparently simple reaction has been studied in depth over the years and currently all metabolites from ASP intake along with their interaction inside the body are well known[5].

In addition, a literature survey exposed there were numerous analytical methodologies to detect ASP and SA in tablets and biofluids[6-17]. Particularly, for the pharmaceutical industry, the interest raises on the need of developing robust analytical stability methods to ensure drugs will not degrade until the sell-by date has expired[18,19]. Ideally, a stability indicating method (SIM) should be able to distinguish between any degradation product from active substance keeping an accurate and precise quantitation of both[20].

For ASP stability monitoring in tablets, ultraviolet (UV) detector has been found to be fit for the purpose as it is suitable to accurately measure in the microgram order, therefore most of the methodologies reported previously employed high performance liquid chromatography (HPLC) technique[7,13,21-23]. However, with the introduction of multicomponent methods, UV spectrophotometry showed an astonishing capability in the quantitation of compounds in mixtures without previous separation[8-11,15-17,24]. This aspect revealed great advantage over HPLC methods, such as lower cost, less time of analysis and minimum organic solvent waste. This method has been applied for ASP alone or in combination with other drugs, however to our knowledge there is no method employed partial least square (PLS) algorithm. This is peculiarly important because PLS method has become the most common chemometric tool in multivariate analysis.

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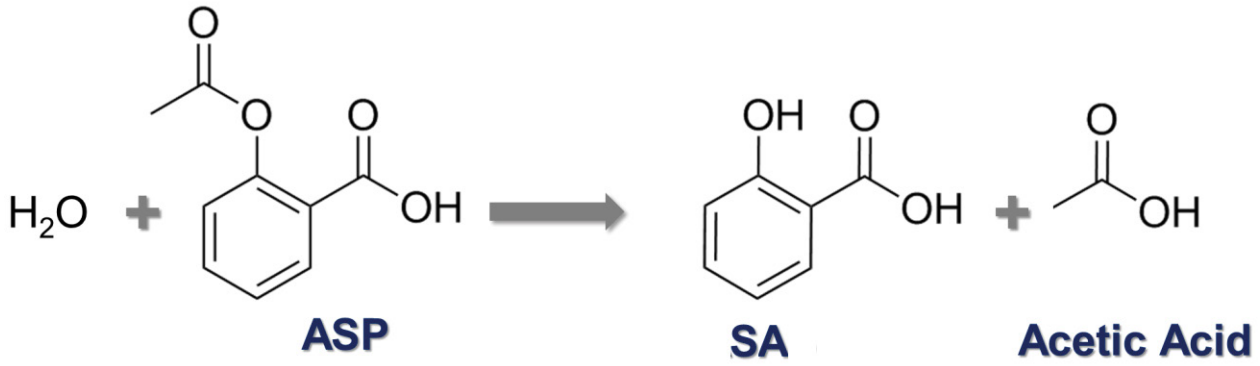

Fig. 1: Principal degradation reaction of ASP.
Aspirin (ASP); salysilic acid (SA)

This is a very robust and reliable tool that allows the simultaneous quantitation of various components in a sample which commonly contains chemical interferences[24]. The PLS model works through a calibration matrix where analytes of interest are mixed in different proportions. The PLS regression model transforms analyte standard concentrations and their resultant absorbance values from a data matrix into latent variables based on a mathematic algorithm, which later predicts the concentration of the analytes from a new sample with high accuracy. Based on the advantages described, the present study shows the development and a full validation of an analytical SIM method using PLS multivariate regression to quantify the content of marketed and developed ASP tablets. This work enhances the repertoire of analytical methodologies employed in the quantitation of ASP in tablets and highlights the advantage of avoiding all kind of separation technique to obtain accurate determinations.

MATERIALS AND METHODS

AAP (100% pure) was purchased from Helm Pureza (Hamburg, Germany) and SA 99.5% was from Sigma-Aldrich (Milan, Italy). Aspirin® commercial tables containing 500 mg of ASP were obtained from Bayer Pharmaceutical Company (Mexico City, Mexico, Batch no. X207UU). Raw material and excipients were obtained from Laboratories "Helm de México" (Mexico) and anhydrous alcohol reagent grade was from J.T. Baker. Anhydrous alcohol was the solvent used for all the solutions preparation, since it is well known for the hydrolysis of ASP on aqueous media.

A double-beam system Cary Win UV/Vis, model Cary 1 E Scanning Spectrophotometer (Varian, California, USA), equipped with 1 cm quartz cell was used. Absorption spectra and simple runs were recorded over the wavelength range of 200-350 nm against the blank using anhydrous alcohol. All the measurements were obtained at room temperature and ambient humidity. Data was recollected using the programs 'Cary simple reads' and 'Cary scan' in ASCII format.

Calibration mix:

Standard solutions for the calibration mix were prepared from independent stock solutions of ASP and SA (25 mg/100 ml) in anhydrous alcohol. Standard series of solutions containing ASP from 30 to 70 µg/ml and SA from 0 to 40 µg/ml were obtained accomplishing a combination set of 13 mixture systems by triplicate (fig. 2). The calibration solutions were prepared freshly every day from stocks.

PLS multivariate regression method:

The mathematical PLS multivariate algorithm is detailed elsewhere[25]. Basically, in order to apply the PLS regression, a calibration matrix containing standard binary solutions has to be performed. These solutions have to be measured at different wavelengths, and their absorption has to be noted on a data matrix. These data is pretreated by resting the average of the concentration and absorbance at each wavelength to each original data, this step is called centring and normalization. Using this transformed matrix, the PLS algorithm is applied; hereby the resultant regression algorithm creates a PLS model that might estimate the concentration of analytes in unknown samples. The quality of the resultant model is assessed by the prediction capability (R^2). The complete description of this methodology is presented in fig 2. Multivariate data treatment was carried out by Datan 5.0.4.4 statistical program (Copyright Multid Analyzes AB).

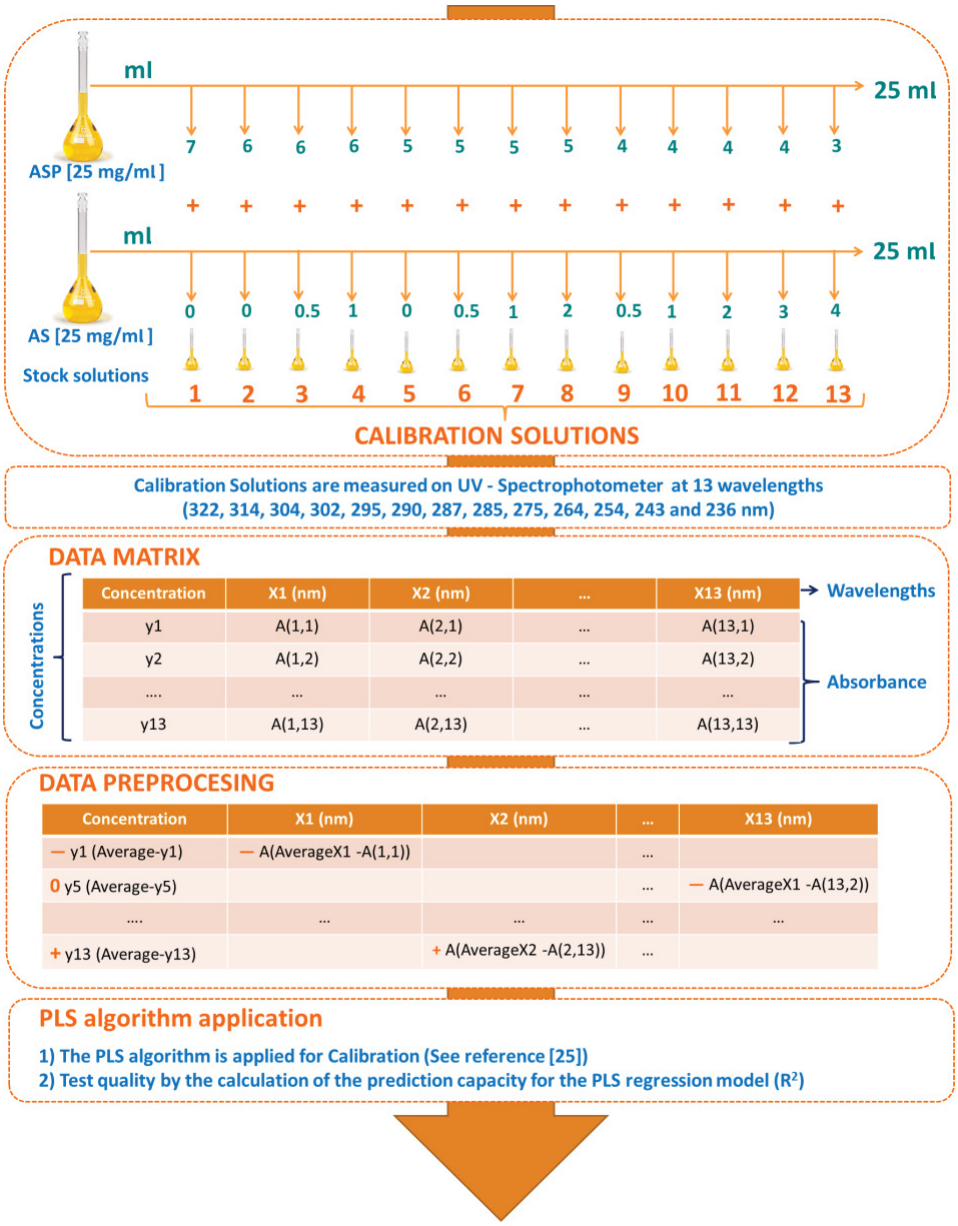

Fig. 2: Workflow of calibration matrix.
The model obtained from the last step is used to calculate unknown concentrations of ASP and SA on new samples.

Sample preparation:

Twenty tablets were weighed and powdered in a mortar until a uniform powder was obtained. Approximately 60 mg of tablet containing the equivalent amount of 50 mg of ASP was weighed and transferred to a 100 ml beaker where a volume of 60 ml of anhydrous alcohol was added. The sample was dissolved and stirred with magnetic agitation for 5 min at 60 rpm. After complete dissolution, the sample was filtered using Whatman 40 filter paper. The filtrate was collected and filled up to the mark in a 100 ml volumetric flask using anhydrous alcohol (solution A). Second dilution was performed using an aliquot of 5 ml from solution A, and diluted with anhydrous alcohol to the mark in a 50 ml volumetric flask (solution B). Resulting solution was in a concentration of 50 µg/ml.

Validation study:

The UV/Vis spectrophotometry methodology using PLS multivariate regression, was validated accordingly to ICH guidelines[26,27] with emphasis on specificity, linearity, precision, accuracy, sensitivity, tolerance and robustness. In particular, specificity was demonstrated by comparing the slope of a calibration curve using standards and spiked samples and through the significant analysis using analysis of

variance (ANOVA) of concentrations sets obtained by interpolation of values from one calibration curve on the complementary curve and vice versa.

Linearity was estimated by assaying at least five levels of concentrations in mixture of ASP and SA in triplicate, covering values ranges from 60 to 140% for ASP (30-70 µg/ml) and from 0 to 80% for AS (0-40 µg/ml). For samples, spiking was performed in 5 different proportions of ASP:SA (110:0, 100:0, 90:10, 80:20, 70:30%), respectively. Precision and recovery were checked by injecting mixture solutions of ASP and SA standards and spiked samples (120:10, 100:0, 80:40% of ASP:SA) by sextuple. Within-day and inter-day precision was evaluated on the spiked samples prepared at the 100% ASP of linearity by two analysts on different days. Furthermore, sensitivity was estimated using 7 levels of ASP and SA very close to each other in concentration by triplicate using standards whereas 9 levels were proposed for spiked samples. Sensitivity was calculated using least significant difference (LSD) statistical test.

Tolerance was estimated measuring three replicates of ASP 100% in spiked samples at the established wavelengths and 0.2 units above. The robustness was also determined by estimating the influence by the change of medium dissolution to methanol. In this case, absorption spectra of ASP and SA were obtained to observe any change in the systems.

ASP degradation after sample preparation:

Degradation of prepared samples was checked over the time using standard binary solutions, developed and Aspirin® marketed tablets. For standards, six replicates of ASP:SA standards mixes (120:10, 100:0, 80:40% of ASP:SA ratio) were analysed every 3 h until 22 h after sample preparation.

RESULTS AND DISCUSSION

Information about ASP and SA was obtained from absorption spectrum of each compound alone and mixed, all dissolved in absolute alcohol (fig. 3). The results showed there was a strong overlapping in almost all wavelengths of the spectra. Therefore on a traditional way of analysis, a direct determination of ASP and SA in a pharmaceutical sample could not possible without a previous separation of analytes. Consequently multivariate regression was tested as an excellent choice to achieve the quantitation of both drugs without the necessity of separation equipment.

The wavelength selection for the PLS regression was done choosing those wavelengths with greater sensitivity to concentration and those where analyte showed a characteristic absorption behaviour in the spectrum compared to other compounds of the mix including excipients. Following these criteria (fig. 3), wavelength range from 200 to 236 nm was avoided, because over this range it is very likely to observe an interaction with any excipient. From 236 to 295 nm range, ASP showed higher absorption than SA, and had a maximum signal at 275 nm. Therefore, this range was optimum to select some wavelengths for the model. The range from 295 to 350 nm only SA showed signal with a maximum at 304 nm hence some wavelengths were also selected at this range. Herein, continuous mathematical simulations were performed; this means that PLS algorithm was applied to different data matrix, at first using all absorbance wavelengths from the spectrum, and then applying the criteria previously commented. The final regression PLS model was set until the most reliable and straightforward model was obtained considering minimum number of wavelengths. This optimization was based on the accuracy of the PLS to predict the real concentration of standard solutions containing ASP and SA, and checking there was no bias in the estimation. After optimization, 13 was the minimum number of wavelengths for the calibration matrix, these were; 322, 314, 304, 302, 295, 290, 287, 285, 275, 264, 254, 243 and 236 nm. Of note, in the first four wavelengths ASP does not showed absorbance, which meant that for single quantitation of ASP, it would be necessary only 9 wavelength measures.

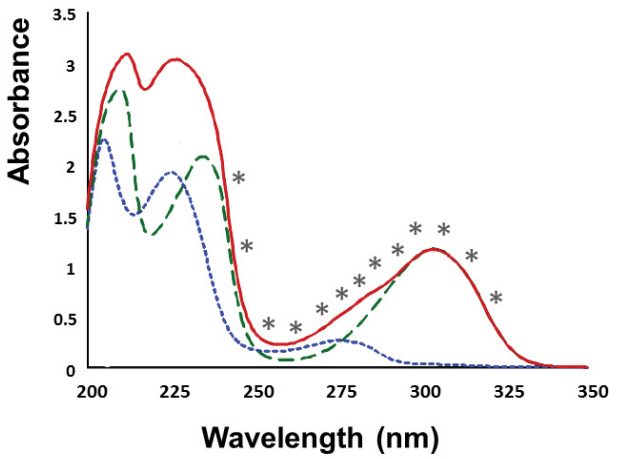

Fig. 3: Comparison of absorption spectra in anhydrous alcohol media.
A) Aspirin 32 µg/ml (ASP), B) salicylic acid 32 µg/ml (SA) and C) standard mixture (ASP+SA). *Selected wavelengths for ASP and SA calibration curves. — — — ASP; — — — SA; ——— ASP+SA

Another important factor for the PLS regression model is the number of binary solutions needed for the matrix calibration, the initial number was set considering all the possible combinations and levels where ASP and SA can be found mixed, this is necessary in order to confer discriminating power to the algorithm. The initial number of calibration binary solutions was set at 39 and was optimized up to 13 solutions. The results from both sets of solutions were compared, showing non statistical significance difference after ANOVA test, in the recovery of three levels of ASP+SA standard mixes prepared by sextuple. Finally, calibration matrix used for the PLS model involved only 13 standard solutions, measured at 13 wavelengths. The statistical parameters of the PLS multivariate method showed 4 as the best number of latent variables, this was calculated based on the linearity of the model by the prediction sum of squares (PRESS). Importantly, the goodness-of-fit statistics of the final PLS model showed a good prediction capability of R^2=0.9984 and a standard error of the regression of S=0.1284 for ASP. Additionally, in order to prove reproducibility, and not hydrolysis of ASP at room temperature and ambient humidity conditions, the calibration mix was evaluated for five consecutive days. Results showed that there were no statistical significant differences after ANOVA test on the quality parameters of the models.

Once spectrophotometric PLS conditions and sample treatment were established, the method was validated following ICH guidelines[25,26]. Selectivity was checked by comparing the analysis of a standards mixes, spiking samples and placebo. It was observed that placebo showed some absorbance at the range of 200-236 nm. However, this aspect did not interfere with the method since this wavelength range was not considered in the model.

As first test, two calibration matrixes were performed using either standards or spiked samples. The results showed negligible difference (0.02%) in the slope value between both calibration curves. Moreover, recovery calculation based on interpolation of the systems from one curve using the complementary calibration matrix showed no statistical differences on recovery after ANOVA test (mean recovery=99.41% and 98.96% of ASP using standard and spiked sample curves, respectively).

Results from the proper parameters of the validation are shown on Table 1. Regarding linearity, both standards and samples showed a good linear regression with determination coefficients over 0.99 and no bias. The %RSD of slopes for ASP and SA ranged less than 0.75% for both, representing the good fit of individual points to the regression line. Method precision had RSD values of 0.43 and 1.16% for ASP using standards and samples, respectively. Precision at first day for spiked ASP at 100%, proved RSD values of 0.94 and 0.38%

TABLE 1: SUMMARY OF VALIDATION PARAMETERS FOR ASP*

Linearity and range from standards (n=13)					
Standards	**r^2**	**A±SD**	**B±SD**	**Range µg/ml**	**%RDS§**
ASP	0.9997	0.9997 (±0.0032)	0.014 (±0.1632)	30-70	0.38
SA	0.9999	0.9999 (±0.0012)	0.0012 (±0.0285)	0-40	0.75
Samples					
ASP	0.9963	0.9926 (±0.0166)	0.1585 (±0.6732)	36.5-56.5	0.50
Precision and Recovery (n=6)					
Standards	**Mean recovery (%)**	**Recovery SD**	**Precision, RSD(%)**		
ASP	99.41	0.42	0.43		
SA	99.75	0.61	0.61		
Samples					
ASP	98.96		1.16		
Inter-day and Inter-analyst precision of ASP mean recovery (%)					
	Repeatability Day 1 (n=3)	**Intermediate precision Day 2 (n=3)**	**Inter-day repeatability Day 1+2 (n=6)**		
Analyst 1	100.82	99.26	100.04		
Analyst 2	99.21	101.92	100.56		
Inter-analyst repeatability (n=6)	100.01	100.59			

*A, slope (±standard error); B, intercept (±standard error); §: predicted concentration/real concentration (%RSD)

by two analysts while 1.05 and 0.85% were obtained from two days of analysis. Therefore, precision met the expected values to be validated for inter-analyst and inter-day tests. Moreover, recovery was found in 100.01% and 100.59% of ASP for standards and samples, respectively, accomplishing validation criterion.

Sensitivity proves how precise is the method to discriminate small differences in the analyte concentrations. Moreover, in the case of degradation products for which the amount is frequently very small and the acceptable limits are reduced; sensitivity becomes paramount interest to decide whether the pharmaceutical product is still in good condition to be sold. Results for sensitivity system showed that it can discriminate concentrations of 4 and 1 µg/ml for ASP and SA, respectively, whereas in samples the sensitivity was 1.5 and 1.8 µg/ml for ASP and SA. These results demonstrated high sensitivity of the analytical method to quantify accurately close concentrations.

Tolerance was measured 0.2 nm above established wavelengths in six spiked samples containing 100% of ASP, where no statistical difference was observed after two way ANOVA test (P=0.11). Moreover for robustness, absorption spectra were obtained using methanol as dissolving reagent. The results showed similar absorption patterns to anhydrous alcohol. No statistical difference was recorded for recovery of spiked samples dissolved in methanol compared to anhydrous alcohol (P=1.0 for both analytes).

The last point in the validation method was the stability study of the already prepared standards and tablet samples. Therefore, prepared standard binary solutions were measured fresh and after different time points, always maintained at room temperature (Table 2). The results showed that the ASP recovery did not change until 6 h after the standard mix was prepared, obtaining a mean recovery of 96.61% and a RSD of 0.98%. This is important as 6 h is the lapse time where ASP did not suffer any degradation and can be measured accurately using the model from the PLS regression. Furthermore, stability of developed and Aspirin® commercial tablets was tested through six replicates from each. Results in Table 2 proved SA started to appear after the first hour in Aspirin® samples whereas for developed tablets ASP still stable until 2 h after sample was prepared. Thus, after 6 h of storage, an average of 3.7% of SA is formed in Aspirin® samples.

The results of this method showed great advantages over those techniques that use previous separation of the compounds. Herein, this method is low cost, easy to carry out and to get established as a routine analytical method. Once the calibration matrix is set, uses short time of analysis and low organic volume compared to HPLC technique, and the analytical response of

TABLE 2: APPLICATION OF THE STABILITY INDICATING METHOD FOR ASPIRIN AND SALICYLIC ACID IN STANDARD SOLUTIONS, SYNTHETIC AND ASPIRIN® COMMERCIAL SAMPLES

[a]Standard ASP+SA (n=18)	ASP		SA	
h	$\overline{X}$ recovery (%)	RSD (%)	$\overline{X}$ recovery (%)	RSD (%)
0	99.3	0.43	101.2	0.92
3	97.8	0.89	111.4	2.04
6	96.6	0.98	115.2	1.24
22	92.6	0.73	136.7	0.51
ASP	**[b]ASP developed (n=6)**		**[c]ASPIRIN® (n=6)**	
h	$\overline{X}$ recovery (%)	RSD (%)	$\overline{X}$ recovery (%)	RSD (%)
0	99.3	0.40	98.7	0.77
1	98.3	0.69	96.2	0.82
2	96.4	0.88	95.6	0.21
4	92.9	1.42	94.1	0.78
6	91.9	1.25	91.1	0.66
SA formation	**[b]ASP developed (n=6)**		**[c]Aspirin® (n=6)**	
h	$\overline{X}$ recovery (%)	RSD (%)	$\overline{X}$ recovery (%)	RSD (%)
0	0	0	0	0
1	0	0.42	0.39	0.24
2	0.83	0.43	1.01	0.29
4	2.63	0.48	2.11	0.24
6	4.54	0.72	3.72	0.37

ASP and SA perfectly suits with the purpose of the determination.

This paper demonstrates in depth the high potential of PLS regression in the development of a stability-indicating method resolving overlapped absorbance spectra in binary unbalanced mixes containing high concentration of ASP and very low quantity of SA. Noteworthy, this method is able to quantify accurately both compounds without prior separation avoiding the use of extra equipment. It is fast and easy to perform, and adaptable as a routine analysis at low cost. This methodology represents a reliable alternative from existing methods to analyse ASP in tablets.

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