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Comparison of assay methods by second-derivative spectroscopy, colorimetry and fluorescence spectroscopy of salicylic acid in aspirin preparations with a high-performance liquid chromatographic method

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Abstract: Second-derivative spectroscopy, colorimetry and fluorescence spectroscopy have been compared with a high-performance liquid chromatographic (HPLC) method for the assay of salicylic acid in preparations of aspirin. Results are presented for the linearity, sensitivity and reproducibility of these methods. The second-derivative spectroscopic and the HPLC methods were acceptable in terms of linearity, sensitivity and inter-day reproducibility and were convenient for the routine analysis of salicylic acid in aspirin preparations.

Keywords: *High-performance liquid chromatography; second-derivative spectroscopy; fluorescence spectroscopy; colorimetry; assay methods for salicylic acid.*

Introduction

Salicylic acid (AS) is the major decomposition product of acetylsalicylic acid (AAS). It is well known that the free acid can cause gastric diseases; the limit of salicylic acid content in aspirin tablets is prescribed to be 0.3% for conventional tablets by the United States Pharmacopeia and the British Pharmacopoeia [1, 2]. Therefore, several methods have been developed for its determination in AAS pharmaceutical preparations. Colorimetric methods such as those of Trinder [3, 4] and McNally [5] have been widely used. Other available methods are: thin-layer chromatography (TLC) [6, 7], second-derivative spectroscopy [8], fluorimetry [9–11], voltammetry [12], gas–liquid chromatography (GLC) [13] and high-performance liquid chromatography (HPLC) by normal-phase [14] and reversed-phase [15, 16] techniques.

This study compares different methods for the assay of AS in aspirin preparations: colorimetry, the official method of the USP and BP for the assay of aspirin powder [1, 2], second-derivative spectrometry, fluorimetry, and high-performance liquid chromatography (HPLC), which is the official method of the USP for aspirin tablets [1].

Experimental

Materials

Acetylsalicylic acid (AAS) USP, salicylic acid (AS) PRS, potassium chloride, potassium biphthalate, sodium hydroxide, absolute ethanol, iron (III) nitrate and mercury (II) chloride were purchased from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from Probus. Glycine was purchased from Sigma (St Louis, MO, USA). Distilled de-ionized water was used for the preparation of all aqueous solutions.

Equipment

Hewlett–Packard HP-1082 HPLC. Beckman DU-6 spectrophotometer. Perkin–Elmer 204 fluorimeter.

Chromatographic conditions (HPLC)

The mobile phase was ortho-phosphoric acid (20 mM)–potassium hydroxide (pH 3.5; 0.1 M) in water–methanol (75:25, v/v). The mobile phase was filtered through a Millipore

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filter (0.45 μm) prior to use. A Hewlett-Packard C_{18} column (10 μm particles, 200 × 4.6 mm i.d.) was used; the flow-rate was 1.5 ml min^{-1}. Injection volumes were 50 μl and ultraviolet detection at 254 nm was used.

Second-derivative ultraviolet spectrometric conditions

The derivative spectra were obtained at a slit width of 8 nm and a scan speed of 150 nm min^{-1}. Chloroacetic acid (1%) in ethanol was used as a solvent. All the samples were assayed in less than 20 min after preparation to avoid possible degradation.

Trinder colorimetric conditions

The Trinder reagent was iron (III) nitrate 4 g, mercury (II) chloride 4 g hydrochloric acid (1 M) 12 ml and water to 100 ml. Reagent solutions were refrigerated and used within 15 days of preparation. Half a millilitre of each of different standard AS solutions were added to 4.5 ml of Trinder reagent and these solutions were centrifuged for 10 min. The absorbance of the solutions was measured at a maximuim of 540 nm.

Fluorimetric conditions

The fluorescence of AS was determined at the maximum excitation and emission wavelengths of 305 and 410 nm, respectively. Slits (10 μm) in bandwidth were fixed. Sensitivity was 6 and selectivity was X_{10}. Chloroacetic acid (1%) in ethanol was used as the solvent. All the samples were assayed within less than 10 min after preparation to avoid possible degradation.

Calibration curves for AS

Working solutions containing 0–100 μg ml^{-1} AS were prepared just before assaying different standard solutions containing 10^{-4}–10^{-5} μg ml^{-1} of AAS.

Treatment of analytical data

The gradients, intercepts of the calibration curves, and the linearity of each calibration graph were obtained by regression analysis. The relative retention time was calculated and also the real retention time [1]. The resolution between two chromatographic peaks (R) was calculated from equation [1] as follows:

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}, \qquad (1)$$

where t_1 and t_2 are the retention times and W_1 and W_2 the width of the peaks, measured by extrapolating the relatively straight sides to the baseline.

Detection limits (DL) were statistically calculated from the following equation [17]:

$$DL = \left(S_0^2 \frac{n-2}{n-1}\right)^{1/2} \cdot \frac{t_p}{b} \qquad (2)$$

where n is the number of values, t_p is the value of Student t at $P = 0.05$ level of significance and $(n - 2)$ degrees of freedom, b is the gradient and S_0^2 is the variance characterizing the dispersion of the points with respect to the line of regression.

The limit for experimental detection is the lower concentration that can be found.

The analytical recovery was calculated from 100 × amount found/amount added at these concentrations.

The intercept value was calculated by the following equation:

$$a \pm t\, S_a \qquad (3)$$

where t is the value of Student t for $n - 2$ degrees of freedom, $P = 0.05$; and S_a is the variance of the intercept value. If the zero value is between these limits, the proportionality condition is achieved.

The confidence intervals for the slope of the line of regression were calculated by the following equation:

$$b \pm t\, S_b \qquad (4)$$

where t is the value of Student t for $n - 2$ degrees of freedom, $P = 0.05$, and S_b is the variance of the slope.

Linearity was evaluated by the relative standard deviation of the slope according to the following equation:

$$S_{b\ rel\ (\%)} = \frac{S_b}{b} 100 \qquad (5)$$

Results and Discussion

Figure 1 shows the HPLC chromatograms of AS and AAS with relative retention times of 0.61 and 1.0, respectively. The values are similar to the relative retention times of the USP [1]. The resolution between chromatographic peaks (R) for AS and AAS was 2.2.

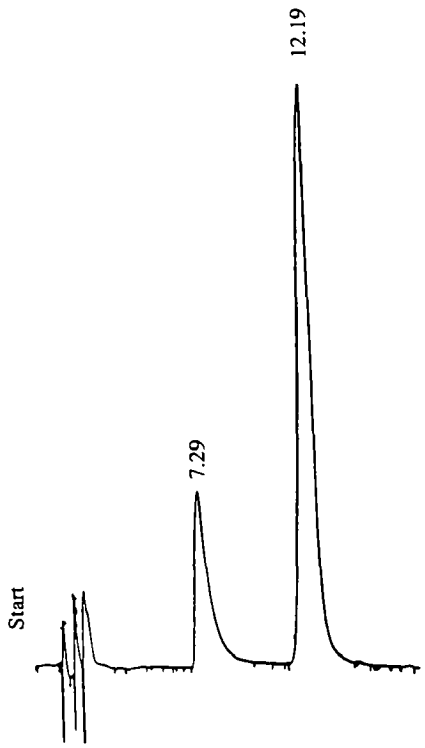

Figure 1
Representative chromatograms of salicylic and (AS) and aspirin (AAS). Relative retention times: 0.61 (AS) and 1.0 (AAS).

This value allows good resolution without interference from AAS in the analysis of AS.

The HPLC method, the official method of the USP [1] was chosen as the analytical reference method. Second-derivative spectroscopy, colorimetry and fluorimetry were compared with HPLC.

The gradients, intercepts and linearity of each calibration graph were calculated and are summarized in Table 1. The negative or positive intercept values for HPLC, colorimetry and fluorimetry were not statistically ($P < 0.05$) different from zero. The second-derivative spectroscopic method gave intercept values statistically ($P < 0.05$) different from zero. This fact probably influenced the higher detection limit values found. Calibration curves for these methods were linear in the ranges tested. The order of linearity for these methods was: colorimetry > second-derivative spectroscopy > HPLC. The fluorimetric method gave poorer linearity at low (<25%) and high concentrations (>85%) in the ranges tested (0–100%). The poorer linearity for the fluorimetric method was probably due to the slit used for measurements and the poor stability of the 1% chloroacetic acid–ethanol solution (3% RSD for the same sample after 10 min of assay).

The concentration range, detection limit and relative sensitivity are summarized in Table 2.

The detection limits evaluated by the statistical method were similar to those calculated according to the experimental method. The lowest detection limit calculated was obtained for the fluorimetric method (0.78 μg ml^{-1})

Table 1
Comparative analytical data for the determination of salicylic acid

Analytical methods	Slopes* $b \pm t\,S_a$ (cm^{-1} μg ml^{-1})	Intercept† $a \pm t\,S_a$	Linearity $S_{b\,rel}$ (%)
HPLC	10.60 ± 0.88	−12.248 ± 17.210	2.60
$^2D_{326}$	1.73 ± 0.12	−8.150 ± 3.22	2.25
Colorimetric	0.0085 ± 0.00049	−0.012 ± 0.26	1.82

*Confidence intervals of the slopes ($P < 0.05$).
†Confidence intervals of the intercept values ($P < 0.05$).

Table 2
Concentration range, detection limit and relative sensitivity for the different methods

Analytical methods	Concentration range (μg ml^{-1})	Detection limit (μg ml^{-1})		Relative sensitivity*
		Calculated	Found	
HPLC	0–40	1.27	1.25	1.0
$^2D_{230}$	0–40	1.93	2.25	1.52
Colorimetric	20–100	3.02	3.25	2.38
Fluorimetric	0–10	0.78	0.50	0.61

*Calculated relative to the HPLC method.

indicating the highest sensitivity. A similar value has been reported previously by Veresh [18]. The colorimetric method was the least sensitive (3.02 $\mu g\ ml^{-1}$). Relative sensitivities, based on detection limits, were calculated with respect to the chromatographic method. The order of sensitivity for these methods was: fluorimetry > HPLC > second-derivative spectrometry > colorimetry.

The difference between the detection limits, "calculated" and "found", for the second-derivative spectroscopic method is probably due to the intercept values which are statistically ($P < 0.05$) different from zero.

The inter-day reproducibility ($n = 3$) of these methods was determined by the RSD obtained at different AS concentrations (Table 3).

The methods of HPLC and second derivative spectroscopy were reproducible with RSD values not exceeding 2.1%. The RSD for AS in aspirin preparations was not more than 4.0% for the USP XXII method [1]; similar results were obtained by Das Gupta (RSD <4.8%) [19]. Poor reproducibility of the fluorimetric (RSD 6.1%) and colorimetric (RSD 5.8%) methods was shown and this fact was a limiting factor in the quantification of AS for laboratory control use; the RSD of AS in aspirin preparations was not more than 4.0% for the USP XXII method [1]. Under the experimental conditions described, HPLC and second-derivative spectroscopy were the methods preferred for the assay of AS in samples with high AAS concentrations. The correlation between these two methods was linear.

The analytical recoveries at these low, medium and high concentrations were 99.1, 104.1 and 103.7%, respectively, for HPLC, and the 102.1, 97.5 and 104.4%, respectively, for second-derivative spectroscopy.

The HPLC and the second-derivative spectroscopic methods proved to be linear and sensitive with good inter-day reproducibility and were convenient for the routine analysis of AS in AAS samples.

Commercially available tablets were analysed by the HPLC and second-derivative spectroscopic methods. The results obtained are summarized in Table 4. No significant differences were found between the results obtained by HPLC and second-derivative spectrophotometry for the same batch, at the 95% confidence level (Student *t*-test and *F*-Snedecor).

The % AS limit in conventional tablets of AAS was 0.3% by the USP XXII method [1] and the BP 1988 method [2]. This proportion was higher than that of AS found in conventional tablets with a long commercial life, where the proportions were 0.239% (HPLC) and 0.254% (second derivative spectroscopy) (batch B). The amount of AAS in all batches was within the content limits of AAS 90.0–110.0% in the USP XXII [1] and 95.0–105.0% in the BP 1985 [2]. The analytical results for the commercial tablets indicate that they comply with the USP and BP specifications for AS and AAS.

Table 3
Inter-day reproducibility ($n = 3$) for the different methods of determination of AS with different standards of AAS

Analytical methods	Standard AAS* $\mu g\ ml^{-1}$	AS ($\mu g\ l^{-1}$)	AS (%AS)†	RSD (%)
HPLC	4×10^4	10	(0.025%)	0.91
		20	(0.050%)	1.81
		30	(0.075%)	2.06
$^2D_{236}$	4×10^4	10	(0.025%)	1.59
		20	(0.050%)	1.99
		30	(0.075%)	1.45
Colorimetric	1×10^5	40	(0.040%)	5.27
		60	(0.050%)	5.77
		80	(0.080%)	3.07
Fluorimetric	1×10^4	2	(0.020%)	5.24
		5	(0.050%)	5.60
		8	(0.080%)	6.14

* AAS concentrations required to assay AS concentrations close to 0.1% (w/w).
† (%AS) AS percentage in relation to AAS concentration.

Table 4
Assay results for the determination of salicylic acid (AS) and aspirin (AAS) in a laboratory mixture and in commercial tablets*

	Recovery (mean ± SD)†			
	AS		AAS	
Sample	HPLC	$^2D_{236}$	HPLC	$^2D_{236}$
Laboratory mixture	0.078 ± 0.016 F = 2.0665 t = 1.6152	0.062 ± 0.023	103.07 ± 0.91 F = 1.4337 t = 2.3617	102.08 ± 0.76
Commerical tablets				
(Batch A)	0.082 ± 0.015 F = 1.6044 t = 1.6152	0.098 ± 0.019	102.06 ± 0.82 F = 1.3994 t = 0.9575	101.63 ± 0.97
(Batch B)	0.239 ± 0.020 F = 1.2346 t = 1.5767	0.254 ± 0.018	100.97 ± 0.92 F = 1.1995 t = 0.6584	102.68 ± 0.84

* Each commercial conventional tablet contained 500 mg of AAS. Batch A was kept for 4 months before the analysis. Batch B was kept for 5 years before the analysis.
† Mean and standard deviation for five determinations.

Conclusions

The values for the detection limits evaluated by the statistical method were similar to those for the detection limits found by the experimental method. The linearity, sensitivity and reproducibility of these methods were obtained.

Under the experimental conditions described the linearity was best in colorimetry > second-derivative spectroscopy > HPLC > fluorimetry. Fluorimetry was the most sensitive method followed by HPLC > second-derivative spectrophotometry > colorimetry. Inter-day reproducibility, expressed as the relative standard deviation (RSD), was always lower than 6.1% and the best reproducibility was for HPLC > second-derivative spectroscopy > fluorimetry > colorimetry. It can be concluded that the HPLC and second-derivative spectroscopic methods were linear with good inter-day reproducibility and with good sensitivity for the routine analysis of salicylic acid (AS) in pharmaceutical preparations of acetylsalicylic acid (AAS).

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