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Analysis of thiamphenicol glycinate salts in pharmaceutical formulations by derivative UV spectroscopy

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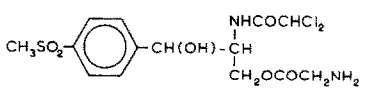
Summary

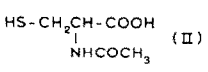
Assay procedures based on second-order derivative UV spectroscopy have been developed for the determination of thiamphenicol glycinate salts in pharmaceutical formulations. The different assay parameters, linearity and precision of the methods have been assessed. The procedures proved to be suitable for a rapid and reliable quality control of commercial pharmaceutical formulations containing the glycine ester of thiamphenicol in different salt forms. When thiamphenicol glycinate (antibiotic) was associated with *N*-acetyl-L-cysteine (mucolytic agent) or with 4-hydroxyisophthalic acid (aspirin-like compound) in salt form, the proposed method allowed a simultaneous determination of both the active salt components.

Introduction

Thiamphenicol glycinate, (2R,3R)-2-[(dichloroacetyl)amino]-3-hydroxy-3-[4-(methylsulfonyl)phenyl]propyl glycinate (I), is an antibiotic with a broad spectrum of activity resembling that of chloramphenicol (Reynolds, 1982; Bowman and Rand, 1980). The drug is useful for the treatment of infections of the respiratory, genitourinary and gastrointestinal systems. Thiamphenicol glycinate is used as the hydrochloride as well as in conjunction with *N*-acetyl-L-cysteine (II) (mucolytic agent) as thiamphenicol glycinate acetylcysteinate or with 4-hydroxyisophthalic acid (III) (antipyretic-analgesic aspirin-like compound) as thiamphenicol glycinate 4-hydroxyisophthalate sodium salt.



(I)



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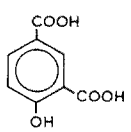

(III)

Few methods have been published for the analysis of the glycine ester of thiamphenicol (I) alone or in pharmaceutical formulations and they include titrimetic (FU IX, 1985; Shukrallah, 1983a and b) and colorimetric (Kanazawa et al., 1970) procedures. The need of more specific analytical methods able to perform a simultaneous determination of both the active compounds in thiamphenicol glycinate salts, led us to regard the

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derivative spectroscopy as a useful technique, owing to its high resolution (Talsky et al., 1978; Fell, 1978), for this topic. So, in the present communication second-order derivative UV spectrophotometric assay procedures are proposed for a rapid and reliable quality control of pharmaceutical formulations containing thiamphenicol glycinate salts.

Experimental

Materials

Thiamphenicol glycinate (TG) hydrochloride and 4-hydroxyisophthalic acid (HPA) were kindly supplied by Zambon S.p.A., Italy. *N*-Acetyl-L-cysteine (AC) was puriss grade from Fluka AG (Switzerland). All the other chemicals were RPE grade from C. Erba (Italy). The commercial samples were dried in vacuum at ambient temperature for 3 h before the analysis.

Instruments

A double-beam Model DMS 90 Varian spectrophotometer, collected with a model 9176 recorder (Varian), was used. The spectra were recorded in 1 cm quartz cells. Suitable settings are: spectral slit width 1.0 nm, scan speed 50 nm/min, chart speed 2 cm/min and recorder span 20 mV full scale. A Crison pH meter and a Mettler M5 microanalytic balance were used.

Thiamphenicol glycinate hydrochloride determination

Calibration curve. Thiamphenicol glycinate hydrochloride stock solution ($c = 1.30$ mg/ml) was prepared in distilled water. The second-order UV spectra of working standard solutions, containing 6.0–55.0 μg/ml of thiamphenicol glycinate hydrochloride, were recorded over the 210–300 nm range against a solvent blank. The calibration curve was then constructed by plotting the measured (mm) amplitudes peak-to-peak $D_{274,278}$ against the corresponding concentrations.

Assay procedure. A sample quantity, equivalent approximately to 70.0 mg of the drug, was dissolved in distilled water to provide an appropriate analytical solution ($c \simeq 28$ μg/ml). The second derivative spectrum of this solution was recorded, the amplitude $D_{274,278}$ was measured (mm) and the drug concentration in the sample was obtained by interpolating the calibration curve.

Simultaneous thiamphenicol glycinate and N-acetyl-L-cysteine determination

Standard solution. A thiamphenicol glycinate stock solution (c = 1.194 mg/ml) was prepared in aqueous sodium hydroxide solution (pH = 11.80) and used to obtain working standard solutions (pH = 11.80) containing 1.9–29.0 μg/ml of the drug. Similarly, *N*-acetyl-L-cysteine stock solution ($c = 0.45$ mg/ml) and working standard solutions ($c = 11.25$–22.6 μg/ml) were prepared in aqueous sodium hydroxide solution (pH = 11.80).

Mixed standard solutions. Into separate 100 ml volumetric flasks, containing 2.5 ml of thiamphenicol glycinate stock solution, were pipetted aliquots of the *N*-acetyl-L-cysteine stock solution to give, after dilution to volume with sodium hydroxide solution (pH = 11.80), mixed standard solutions with acetylcysteine/ thiamphenicol glycinate molar ratios over a 0–2.5 range.

Calibration curves. Second derivative UV spectra of the thiamphenicol standard solutions were recorded and both the measured amplitudes peak-to-peak $D_{274,278}$ and peak-zero D_{236} were plotted against the drug concentrations to obtain the corresponding calibration curves.

For the acetylcysteine determination, the second derivative UV spectra of the mixed standard solutions were recorded, the amplitudes $D_{274,278}$ and $D_{236,266}$ were measured and the $D_{236,266}/D_{274,278}$ ratios were plotted against the respective acetylcysteine/thiamphenicol glycinate molar ratios. A calibration curve for *N*-acetylcysteine alone was constructed based on the amplitude D_{236} measurement.

Assay procedure. An accurately weighed sample quantity, equivalent approximately to 50 mg of thiamphenicol glycinate acetylcysteinate, was dissolved in aqueous pH = 11.80 solution to provide a final analytical solution containing 50 μg/ml of the drug. The second derivative UV spectrum of the resulting solution was then re-

corded and the amplitudes $D_{274,278}$ and $D_{236,266}$ were measured.

The thiamphenicol glycinate concentration was derived from the respective calibration curve ($D_{274,278}$), while the calibration curve from the mixed standard solutions provided the acetylcysteine/thiamphenicol glycinate molar ratio in each sample.

Simultaneous thiamphenicol glycinate and 4-hydroxyisophthalic acid determination

Buffer solution (pH = 4.4). Sodium acetate (136 g) and ammonium acetate (77 g) were dissolved in distilled water (500 ml) and the resulting solution was diluted to 1000 ml with methanol. Acetic acid was then added to obtain a pH = 4.4 value.

Standard solutions. Standard solutions containing 17.0–42.0 μg/ml of 4-hydroxyisophthalic acid were prepared in pH = 4.4 buffer solution. Similarly, mixed standard solutions containing 21.0 μg/ml of 4-hydroxyisophthalic acid and 12.0–56.0 μg/ml of thiamphenicol glycinate were prepared in pH = 4.4 buffer solution.

Calibration curves. 4-Hydroxyisophthalic acid – Second derivative UV spectra of the 4-hydroxyisophthalic acid standard solution were recorded against a solvent blank over the 260–340 nm range and the measured peak-zero amplitude D_{302} was plotted against the drug concentration to obtain the calibration curve. *Thiamphenicol glycinate* – Similarly, the second derivative UV spectra of the mixed standard solutions were recorded and the peak-zero amplitudes D_{302} (specific for HPA) and D_{278} (due to the contributions of both the components) were measured. The individual thiamphenicol glycinate contributions to the amplitudes D_{278} and the corresponding calibration curve were then obtained as described in Results and Discussion.

Assay procedure. A sample quantity, equivalent approximately to 38.0 mg of thiamphenicol glycinate 4-hydroxyisophthalate sodium salt, was dissolved in 100 ml of pH 4.4 buffer solution. After further dilution (1 : 5) with the same buffer solution, the procedure described under calibration curves was then followed. The quantitation of thiamphenicol and 4-hydroxyisophthalic acid was performed by interpolating the respective calibration curves.

Results and Discussion

The absorption (zero-order) UV spectrum of thiamphenicol glycinate hydrochloride exhibits a benzenoid profile with weak absorptions at λ = 266 nm ($A_{1\%,1cm} = 21 \pm 1.5$) and λ = 273 nm ($A_{1\%,1cm} = 18 \pm 1.5$) (FU IX, 1985) which are converted in sharp bands of great amplitude when the second-order UV spectrum is recorded (Fig. 1). In view of this, a second-order derivative UV spectrophotometric method was devised to determine the thiamphenicol glycinate alone as well as combined with *N*-acetylcysteine or 4-hydroxyisophthalic acid in salt form in pharmaceutical formulations. For the thiamphenicol glycinate hydrochloride determination, the amplitude $D_{274,278}$ from second-order spectrum was measured and a linear regression ($y = 26.402c - 3.124$; $r = 0.9999$; $n = 10$) between amplitude $D_{274,278}$ and drug con-

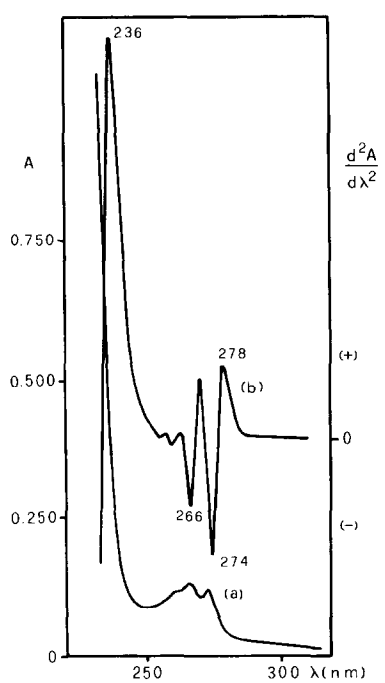

Fig. 1. Absorption (zero-order) UV spectrum (a) and second-order derivative UV spectrum (b) of thiamphenicol glycinate hydrochloride in water ($c = 1.093 \times 10^{-4}$ M).

TABLE 1

Assay results for the analysis of commercial formulations containing thiamphenicol by second-order derivative UV spectroscopy

Drug [a]	Intra-day [b,d]						Inter-day [c,d]					
	TG		AC		HPA		TG		AC		HPA	
	% found	*CV*	% found	*CV*	% found	*CV*	% found	*CV*	% found	*CV*	% found	*CV*
TG-hydrochloride	99.60	0.46					99.86	0.60				
TG-AC [e]	100.16	0.98	99.26	1.25			99.80	1.81	99.62	2.74		
TG-AC [f]	99.55	0.70	100.41	2.51			99.36	2.07	101.0	2.62		
TG-HPA	99.20	1.27			99.50	1.30	99.50	2.30			99.75	1.90

[a] TG = thiamphenicol glycinate; AC = *N*-acetylcysteine; HPA = 4-hydroxyisophthalic acid.
[b] Average of 6 determinations.
[c] Average of 20 determinations.
[d] Expressed as percentage of the label claim.
[e] By graphic procedure.
[f] By equation system (1).

centration over the 6.0–55.0 μg/ml range was obtained. The method was applied to the individual thiamphenicol glycinate hydrochloride determination in a commercial formulation and the assay results are reported in Table 1.

For the simultaneous determinations of thiamphenicol glycinate and acetylcysteine in a thiamphenicol glycinate acetylcysteinate formulation the spectrophotometric analysis was carried out in aqueous sodium hydroxide solution (pH 11.80). Under these conditions, the absorption maximum of acetylcysteine undergoes a bathochromic shift, due to the thiolate anion formation (Kallen, 1971), and the UV spectrum of thiamphenol glycinate acetylcysteinate corresponds to the addition of the individual absorptions from equimolar solutions of thiamphenicol glycinate and acetylcysteine (Fig. 2). In absorption spectroscopy, the properties of thiamphenicol (weak absorption at $\lambda = 266$ nm and 273 nm) and acetylcysteine (absorption maximum at low wavelengths) do not allow a reliable simultaneous determination of the components. Conversely, the second-order derivative UV spectrum (Fig. 3) can constitute the basis for convenient determination of both compounds. The easily measurable amplitude $D_{274,278}$ due to thiamphenicol glycinate appears not to be affected by acetylcysteine, while the amplitude $D_{266,236}$ results from the contributions of both thiamphenicol (positive) and acetylcysteine (negative). Accordingly, when the amplitudes $D_{274,278}$ were plotted against the thiamphenicol concentrations ($c = 5.0$–50.0 μg/ml) a single linear calibration curve ($y = 25.22c - 1.47$; $r =$

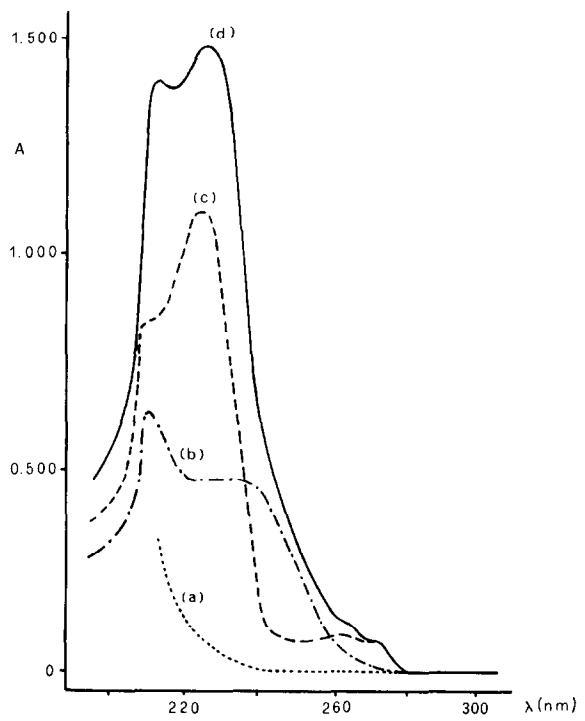

Fig. 2. Absorption (zero-order) UV spectra of equimolar solutions ($c = 1.093 \times 10^{-4}$ M) of: (a) acetylcysteine (water); (b) acetylcysteine (pH 11.80); (c) thiamphenicol glycinate (pH 11.80) and (d) thiamphenicol glycinate acetylcysteinate (pH 11.80).

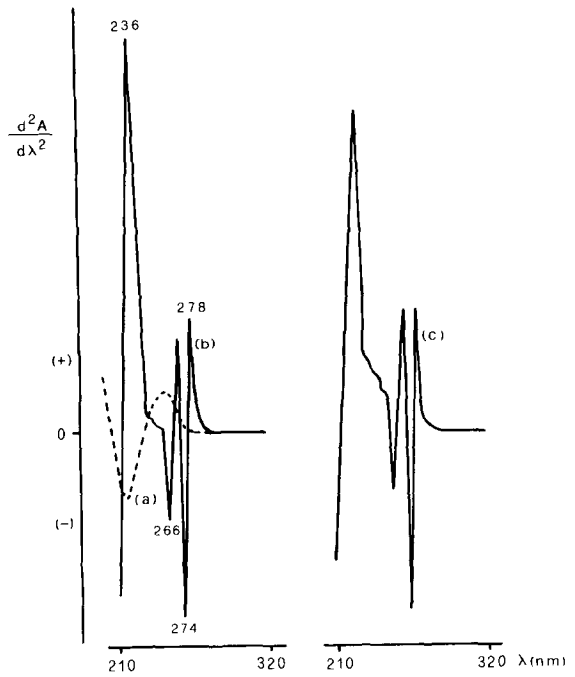

Fig. 3. Second-order derivative UV spectra of equimolar solutions (pH 11.80) of: (a) acetylcysteine; (b) thiamphenicol glycinate; and (c) thiamphenicol glycinate acetylcysteinate. Concentrations as in Fig. 2.

0.9993; $n = 16$) was obtained from thiamphenicol standard solutions and mixed standard solutions.

Conversely, the characteristic $D_{236,266}/D_{274,278}$ ratio ($R = 1.70 \pm 0.01$) of thiamphenicol glycinate, is reduced by the N-acetylcysteine presence (Fig. 3). In effect, a linear plot (slope $= -0.377 \pm 0.003$; intercept $= 1.72 \pm 0.02$; $r = 0.9990$; $n = 7$) was obtained when the $D_{236,266}/D_{274,278}$ ratios, derived from mixed standard solutions, were plotted against the respective acetylcysteine/thiamphenicol glycinate molar ratios over the range of 0–2.5. The results obtained for the assay of thiamphenicol glycinate acetylcysteinate in commercial formulations by this graphic procedure were in good agreement with the labelled claim (Table 1). The data were confirmed by an alternative calculation procedure according to a recently proposed spectrophotometric analysis of two-component mixtures (Korany et al., 1984). The simultaneous determination of thiamphenicol glycinate and acetylcysteine was made by solving the following system:

$$\begin{aligned} D_{274,278} &= C_x\,\delta_{274,278} + C_y\omega_{274,278} \\ D_{236} &= C_x\,\delta_{236} + C_y\omega_{236} \end{aligned} \qquad (1)$$

where $D_{274,278}$ and D_{236} are the amplitudes derived from the sample second-order UV spectrum, C_x and C_y are the concentrations of thiamphenicol glycinate (x) and acetylcysteine (y), δ and ω are the second derivative coefficients D (1%, 1 cm) for the compounds x and y, respectively. The Lambert-Beer law validity in the second-order spectra was verified for both the compounds plotting the selected amplitudes against the respective concentrations. Table 2 reports the parameters and correlation coefficients data for the calibration curves as well as the second-order derivative coefficients D (1%, 1 cm). The reproducibility of the second derivative coefficients δ and ω was found to be satisfactory as indicated by the respective CV values. Introduction of these data into system (1) allowed a simultaneous thiamphenicol glycinate and acetylcysteine de-

TABLE 2

Data for the calibration curves and the second derivative coefficients $D_{(1\%,1cm)}$

Compound	Amplitude [a]	Slope	y-intercept	Correlation coefficient	Range (μg/ml)	δ, ω [b] (1%, 1 cm)	CV
Thiamphenicol glycinate	$D_{274,278}$	25.275	−1.470	0.9993	5–50	23.800	1.10
	D_{236}	33.015	−1.060	0.9996	5–50	31.955	0.90
N-acetylcysteine	$D_{274,278}$	–	–	–	–	–	–
	D_{236}	−15.612	0.073	0.9994	10–30	−15.682	1.61

[a] Measured in mm.

[b] Related to solutions containing 1.0 mg/100 ml of TG (δ) and AC (ω).

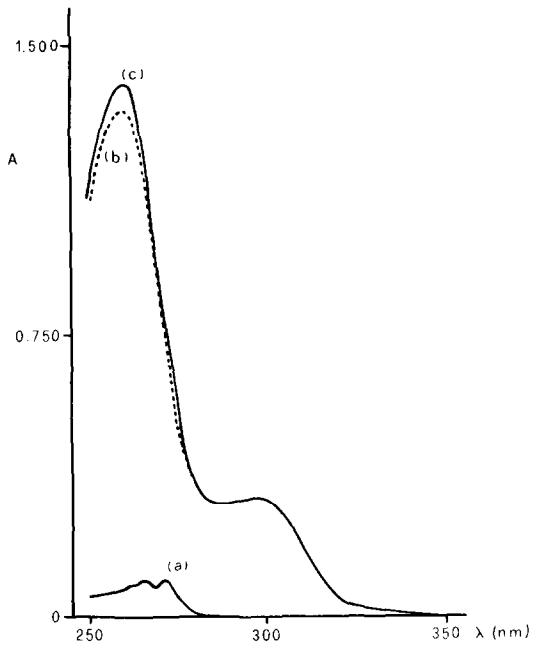

Fig. 4. Absorption (zero-order) spectra of equimolar solutions ($c = 1.093 \times 10^{-4}$ M; pH 4.40) of: (a) thiamphenicol glycinate; (b) 4-hydroxyisophthalic acid; and (c) thiamphenicol glycinate 4-hydroxyisophthalate (equivalent to an equimolar solution of TG and HPA).

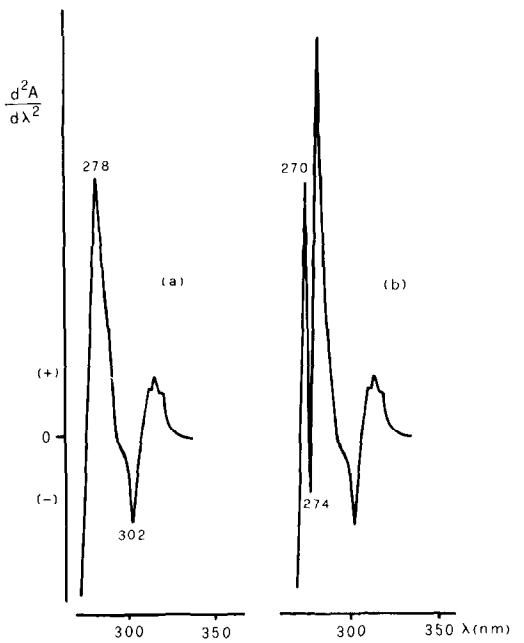

Fig. 5. Second-order derivative UV spectra of equimolar solutions ($c = 1.093 \times 10^{-4}$ M; pH 4.40 of: (a) 4-hydroxyisophthalic acid; and (b) thiamphenicol glycinate 4-hydroxyisophthalate.

termination. Recovery studies on synthetic preparations showed that excipients as EDTA did not interfere with the analysis of both the components.

A second-order spectrophotometric method was devised also for the simultaneous determination of thiamphenicol glycinate and 4-hydroxyisophthalic acid contained in a lyophilized powder as thiamphenicol glycinate 4-hydroxyisophthalate sodium salt. In effect, the addition of an equimolar quantity of thiamphenicol glycinate induces only a slight alteration in the absorption (zero-order) spectrum profile of 4-hydroxyisophthalic acid (Fig. 4), while an increased resolution is achieved when the second-order derivative spectrum is recorded (Fig. 5). The amplitude D_{302} is specific for the 4-hydroxyisophthalic acid and is essentially independent of the thiamphenicol glycinate concentration, while the amplitude D_{278} is due to the contributions from both the compounds. For the specific determination of 4-hydroxyisophthalic acid a linear ($D_{302} = 2.394c - 0.048$; $r = 0.9990$, $n = 8$) calibration curve was obtained ($c = 17.0$–50.0 μg/ml) and used for the compound quantitation. Thiamphenicol glycinate content was then obtained from its specific contribution to the measured amplitude D_{278}. This contribution, D_{278} (TG), was derived as follows:

$$D_{278(TG)} = D_{278(measured)} - K \cdot D_{302(HPA)}$$

where $K = D_{278}/D_{302} = 2.56 \pm 0.03$ for 4-hydroxyisophthalic acid. When the calculated amplitudes $D_{278(TG)}$ obtained from mixed standard solutions were plotted against the corresponding concentrations (5.0–42.0 μg/ml), a linear, $D_{278(TG)} = 1.970c + 0.07$; $r = 0.9993$; $n = 6$, calibration curve was obtained and used for the thiamphenicol glycinate quantitation in the samples. Table 1 shows the assay results for both the components of the pharmaceutical formulation analysed. Recovery studies from synthetic mixtures with varying thiamphenicol glycinate/4-hydroxyisophthalic acid molar ratios showed the method to be satisfactorily accurate (99.0–100.5% recovery) and precise

($CV = 1.2–1.6\%$) for both the components of quantitation. The possibility of using the amplitude D_{270} to achieve a direct and specific quantitation of thiamphenicol glycinate in the presence of 4-hydroxyisophthalic acid was attempted, but some interference resulted in a reduced accuracy.

In summary, the proposed procedures based on second-order derivative UV spectroscopy offer the advantage of an increased resolution and could be used for a rapid and reliable quality control of commercial thiamphenicol glycinate-containing pharmaceutical dosage forms.

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