Colorimetric Determination of Acetylcysteine, Penicillamine, and Mercaptopropionylglycine in Pharmaceutical Dosage Forms

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Abstract □ *A colorimetric determination of acetylcysteine, penicillamine, and mercaptopropionylglycine is described. The method is based on the oxidation of mercapto-compounds with iron(III) in the presence of 1,10-phenanthroline. The iron(II) formed was quantitatively and rapidly converted to the stable tris(1,10-phenanthroline)iron(II) complex measured spectrophotometrically at 515 nm. The results obtained from various commercial formulations indicate that the method proposed allows a simple, sensitive determination of these mercapto-drugs with good accuracy (99.5–101% recovery) and remarkable precision (RSD ±0.6–1.6%).*

Keyphrases □ Acetylcysteine—colorimetric determination in pharmaceutical dosage forms, penicillamine, mercaptopropionylglycine □ Penicillamine—colorimetric determination in pharmaceutical dosage forms, acetylcysteine, mercaptopropionylglycine □ Mercaptopropionylglycine—colorimetric determination in pharmaceutical dosage forms, acetylcysteine, penicillamine □ Colorimetry—analysis of penicillamine, acetylcysteine, and mercaptopropionylglycine in pharmaceutical dosage forms

A number of thiol (SH)-containing drugs are commercially available and are utilized in various therapeutic applications. Among the most widely used are acetylcysteine, a topical pulmonary mucolytic agent; penicillamine, a therapeutic agent for the treatment of Wilson's disease and heavy metal poisoning; and mercaptopropionylglycine, a drug used in hepatic disorders.

Numerous analytical methods have been developed for the quantitative assay of the thiol group (1–3). The USP method (4) for acetylcysteine determination, based on the potentiometric titration of the thiol group with mercuric nitrate using a calomel–gold electrode system, was considered affected by some possible ambiguities due to the divalency of mercury (5). Other procedures reported for determining this drug in pharmaceutical dosage forms include spectrophotometric (6) and colorimetric techniques (5, 7).

Numerous extensive studies on the chelating properties of penicillamine *versus* a variety of metals have been reported (8–19), but analytical procedures for its quantitative determination have been developed more for biological (20–24) than for pharmaceutical systems (25). The official USP method (26) involves the use of the highly toxic mercuric acetate, and recently a study (25) investigated alternative procedures such as nonaqueous amine titration, nonaqueous acid titration, and hydroxylamine colorimetric assay.

A few studies on the chelating ability of mercaptopropionylglycine have appeared (27, 28), but no analytical procedures for its determination in pharmaceutical formulations are available to the authors' knowledge.

It is highly desirable to provide a single colorimetric procedure suitable for all these mercapto-compounds, for which a direct spectrophotometric method is unwarranted due to interference from excipients.

In this report a simple and sensitive colorimetric procedure, based on the formation of the tris(1,10-phenanthroline)iron(II) complex, is described for determining acetylcysteine, penicillamine, and mercaptopropionylglycine in various commercial formulations.

EXPERIMENTAL

Instruments—A digital single-beam spectrophotometer[1], a double-beam spectrophotometer[2], a pH meter[3], and a microanalytical balance[4] were used.

Materials—Pharmaceutical grade acetylcysteine[5], penicillamine[5], and mercaptopropionylglycine[5] were used as working standards. All reagents were analytically pure and were used without further purification. Commercially coated tablets, injectable solutions, syrup of mercaptopropionylglycine, and injectable solutions of acetylcysteine and capsules of penicillamine were analyzed as dosage forms.

Reagents—The 0.25% aqueous solution of 1,10-phenanthroline was prepared by dissolving 0.25 g of the compound in distilled water and diluting to 100 ml after gentle heating. This solution was stored for ≤3 days, in a tight light-resistant container. For the iron(III) 4×10^{-3} *M* solution, 1.92 g of ferric ammonium sulfate dodecahydrate dissolved in distilled water was treated with 10 ml of concentrated hydrochloric acid and diluted with water to a 1000-ml volume. This solution was also protected

Table I—Parameters and Correlation Coefficients of Calibration Plots [a] for Mercapto-Compounds

Mercapto-Compound	*A*	*B*	*r*
Acetylcysteine	10791	−0.001	0.9998
Penicillamine	11100	0.01	0.9999
Mercaptopropionylglycine	11267	−0.001	0.9999

[a] Calibration plots are expressed as regression lines of the form $y = Ax + B$; with y = absorbance, x is the molar concentration of mercapto-compounds, B is the y-intercept, A is the slope of the line; r is the correlation coefficient of the line.

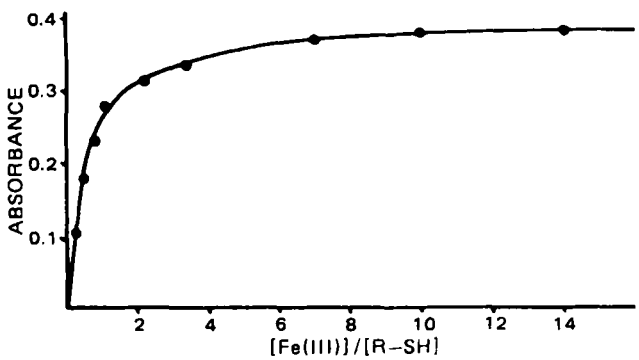

Figure 1—*Absorbance* versus *molar ratio of iron(III) to mercapto-compound (R—SH) for a 0.3 × 10⁻⁴ M acetylcysteine solution, pH 3.4.*

[1] Model UVIDEC 4 Jasco, Japan.
[2] Model 402 Perkin-Elmer Corp., England.
[3] Model 325 Amel, Italy.
[4] Metler type 5, Switzerland.
[5] Fluka, Switzerland.

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Table II—Assay Results of Commercial Pharmaceutical Dosage Forms

Drug	Dosage Form	Declared, mg	Found[a], mg	Recovery, %	*RSD*, %
Penicillamine	Capsules	150	151.0	100.7	0.72
Mercapto-propionylglycine	Ampuls	100	101.0	101.0	1.60
	Syrup	100	99.5	99.5	0.60
	Coated Tablets	250	252.0	100.8	1.50
Acetylcysteine	Ampuls	100	100.1	100.1	0.63

[a] Average of five determinations.

from sunlight. A pH 4 buffer was prepared by mixing 75 ml of 0.1 *M* CH_3COOH with 25 ml of 0.1 *M* CH_3COONa. Whenever necessary, 0.2 *M* CH_3COONa was also used to adjust the pH.

Standard Solutions—For acetylcysteine, 0.163 g of the drug was dissolved in water up to a 500-ml volume; dilution to a 1:5 ratio gave the desired concentration. For penicillamine, 0.149 g of the drug was dissolved in water up to a 500-ml volume, and the solution was diluted five times to give the desired concentration. For mercaptopropionylglycine, 0.167 g of the drug was dissolved in water up to a 500-ml volume; dilution to a 1:5 ratio gave the desired concentration.

Calibration Curves—For calibration of acetylcysteine, penicillamine, and mercaptopropionylglycine serial volumes of 1–5 ml (in 1-ml steps) of standard solution were transferred to 25-ml volumetric flasks. Six milliliters of iron(III) solution, 2.5 ml of phenanthroline, 3.5 ml of sodium acetate (0.2 *M*), and 4.5 ml of buffer and water to volume were added in succession. After 20 min (after 1 hr for penicillamine) the absorbance at 515 nm was measured against a blank, prepared simultaneously without thiol solution.

To evaluate a possible disodium edetate interference, 1–10% of this compound was added to a 3×10^{-5} *M* thiol solution, and absorbance was measured under the described conditions.

Determination of Acetylcysteine—For the determination of acetylcysteine in injectable solution, a volume equivalent to 100 mg of acetylcysteine was diluted to 200 ml with distilled water and 10 ml of this solution was further diluted five times. The procedure for the calibration curve was followed using 2 ml of the final solution.

Determination of Penicillamine—For the determination of penicillamine in capsules, the contents of 10 capsules were mixed, and an amount equivalent to 150 mg of the drug was accurately weighed and dissolved in water to a 500-ml volume. After stirring, the solution was filtered. To 10 ml of the filtrate were added 10 ml of 0.2 *M* CH_3COONa, 15 ml of pH 4 buffer, and water to a volume of 50 ml. Using 3 ml of this solution, the procedure for the calibration curve was followed.

Determination of Mercaptopropionylglycine—For the determination of mercaptopropionylglycine in syrup, 16 mg of powder was dissolved in water up to a 250-ml volume. Using 3 ml of this solution, the procedure for the calibration curve was followed. For the determination of mercaptopropionylglycine in injectable solutions the content of one ampule (2 ml), equivalent to 100 mg of the drug, was diluted with water to 200 ml; 10 ml of this solution was further diluted five times. Using 2 ml of this solution, the procedure for the calibration curve was followed. For the determination of mercaptopropionylglycine in commercial coated tablets, 10 coated tablets were powdered and mixed. A portion of this powder (117 mg) was transferred to a 500-ml volumetric flask, and water was added with stirring for 10 min before filling to volume. Using 3 ml of this solution, suitably filtered, the procedure for the calibration curve was followed.

RESULTS AND DISCUSSION

The proposed method is based on the ability of mercapto-compounds to reduce iron(III) to iron(II) (reaction 1), which is rapidly converted to the highly stable and colored tris(1,10-phenanthroline)iron(II) complex (reaction 2) with λ_{max} = 515 nm and ϵ = 11,100 (29, 30).

$$2\,Fe^{3+} + 2\,R{-}SH \rightarrow 2\,Fe^{2+} + R{-}S{-}S{-}R + 2\,H^+ \quad (1)$$

$$Fe^{2+} + 3\,(C_{12}H_8N_2) \rightarrow [Fe(C_{12}H_8N_2)_3]^{2+} \quad (2)$$

Scheme I

The conditions under which the reaction of each mercapto-compound with iron(III) in the presence of 1,10-phenanthroline fulfills the necessary analytical requirements were investigated. The amount of phenanthroline added must be at least in a 3:1 ratio with respect to the iron(II), according to reaction 2. The time necessary for the completion of the reaction depends on temperature, pH, and on the amount of iron(III) added. At room temperature, which was chosen to meet the practical requirements of analytical speed, the influence of pH and iron(III) concentration was investigated. It was observed that under the described experimental conditions, the optimum pH range for the reaction was 2.5–4.5. This is markedly different from the 7–8 pH range selected previously for the colorimetric determination of cysteine (31) and thioethylamine (32) by means of nitrilotriacetateferrate(III).

The influence of iron(III) concentration on the color development is illustrated in Fig. 1, where the absorbance *versus* molar ratio of iron III to mercapto-compound is reported. By increasing the iron III concentration, the reaction can be forced to completion, as indicated by the constant value of absorbance for iron(III) concentration in up to tenfold excess of mercapto-compound.

All absorbance readings (515 nm) were taken for acetylcysteine and mercaptopropionylglycine after 20 min *versus* the blank, which contained all the reagents except thiol; for penicillamine, color development was found to be complete in longer times (1 hr). In all cases the absorbance was constant for at least 2 hr. Under these experimental conditions, good reproducibility was obtained (*RSD* = 0.34%).

A calibration curve of absorbance *versus* concentration was constructed for each mercapto-compound in the range of 4.5–80 μM. The parameters and correlation coefficients of calibration plots, expressed as regression lines of the form of $y = Ax + B$, are listed in Table I. As can be seen from the equations obtained, the lines pass very close to the origin and the slopes remain relatively constant for all three drugs investigated.

Regression analysis of all of the data by least squares gave the line $y = 11015x + 0.01$ (correlation coefficient of 0.998), which reproduces satisfactorily the experimental absorbance values obtained for various concentrations of all three mercapto-compounds. These results indicate that acetylcysteine, penicillamine, and mercaptopropionylglycine, according to reaction 1, similarly reduce iron(III) to iron(II), which successively forms the phenanthroline complex. The calibration curve obtained for mercapto-compounds was also compared with the calibration plot for iron(II) solutions treated by phenanthroline under the described experimental conditions. The comparison showed that reaction 1 was quantitative, since the absorbance values for the mercapto-compounds and those for iron(II) solutions at the same molar concentration were essentially identical.

The proposed colorimetric method was applied to analysis of commercially available pharmaceutical dosage forms of acetylcysteine, penicillamine, and mercaptopropionylglycine. The results obtained (Table II) indicate that the proposed method is suitable for analyzing various types of formulations. No interference due to air oxidant effect or due to excipients such as magnesium stearate, titanium dioxide, lactose, *etc.* has been observed. The possible interference by disodium edetate salt present in some preparations containing penicillamine and acetylcysteine was investigated. It was found that under the described experimental conditions, concentrations of disodium edetate as high as 10% of the thiol compound did not interfere with the analysis, at variance with some volumetric procedures reported previously (25).

In summary, the proposed method allows a simple, sensitive determination of the thiol-group containing drugs investigated, with good accuracy (99.5–101% recovery) and remarkable precision (*RSD* ± 0.6–1.6%). The fact that this method requires more time in the case of penicillamine than some methods previously proposed (20, 25), is compensated by the high stability of the colored complex and by the lack of disodium edetate interference.

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Arterial and Venous Blood Sampling in Pharmacokinetic Studies: Griseofulvin

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Abstract □ The pharmacokinetics of griseofulvin were evaluated simultaneously using both arterial and venous plasma in three dogs and one rabbit after a rapid bolus intravenous dosing. Initial arterial–venous ratios 20 sec after injection were the highest and ranged from 15- to 752-fold for dogs; the ratio was 3240-fold for the rabbit. Both curves decayed paralleling each other at the terminal phase with the venous levels higher than arterial levels by 14–43 and 8.4% for the dogs and the rabbit, respectively. The use of the instantaneous input principle was found to overestimate the total area under the plasma level–time curve by as much as 166%. An exponential term with a negative coefficient was used to account for the short and steep rising phase of plasma levels after injection. Detailed analyses showed significant differences in various calculated pharmacokinetic parameters based on arterial or venous data. The present study exemplifies the need for careful assessment and interpretation of classical pharmacokinetic parameters. It appeared that short intravenous infusion rather than the instantaneous or rapid bolus intravenous injection should be preferred for routine pharmacokinetic studies.

Keyphrases □ Griseofulvin—pharmacokinetics, arterial and venous blood sampling □ Pharmacokinetics—arterial and venous blood sampling, griseofulvin □ Blood sampling—arterial and venous pharmacokinetics of griseofulvin

Preliminary results showing marked and persistent arterial–venous (A–V) plasma concentration differences of six drugs (propranolol, lidocaine, procainamide, furosemide, theophylline, and griseofulvin), following intravenous administration to dogs or rabbits, were recently reported from this laboratory (1). The pharmacokinetic consequences of data analysis by using arterial or venous data on propranolol also has been described (2).

The present report describes in detail arterial and venous plasma level profiles of griseofulvin in three dogs and one rabbit and discusses the resulting effects on pharmacokinetic analysis.

EXPERIMENTAL

Bolus Injection Studies—Griseofulvin[1] (40 mg/ml) in polyethylene glycol 400[2] (1, 3) was injected as a bolus over 20 sec to the cephalic vein in three male mongrel dogs and to the ear vein in one male New Zealand white rabbit. Dogs 1 and 2 were conscious while dog 3 and rabbit 1 were anesthetized with nitrous oxide during the study. The doses administered to each animal are summarized in Table I. The midpoint of the injection was timed zero. Femoral arterial and venous blood samples were withdrawn simultaneously from permanent cannulas in the dogs and a specially designed T-loop in the rabbit. The preparation of the cannulas and the surgical procedure were described elsewhere (2). Heparinized normal saline (10 U/ml) was used for flushing of the cannula during the study. The sampling times, also midpoints of collection, were usually at 0.33, 0.66, 1, 1.33, 1.66, 2, 3, 6, 9, 15, 30, 60, 80, 100, 120, 140, 160, and 180 min

[1] Sigma Chemical Co., St. Louis, Mo.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.

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