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PHARMACEUTICAL TECHNOLOGY

Automated Fluorescence Analysis of Perphenazine in Dissolution Rate Testing of Tablet Formulations

J. M. KONIECZNY and E. McGONIGLE [x]

Abstract □ An automated fluorescence method is described for determining perphenazine in large numbers of samples, as might be required in dissolution studies. Tablets containing perphenazine and amitriptyline were dissolved in 0.1 *N* HCl. Approximately 5 ml was transferred to the automated system where amitriptyline was removed as the ion-pair by extraction with chloroform and discarded. Perphenazine in the aqueous phase was reacted with permanganate solution to form a fluorophore which is quantitative. No sample blank was required. Analyses may be carried out at a rate of 30/hr. Sensitivity was approximately 0.1 μg/ml with a relative standard deviation of ±1.7%.

Keyphrases □ Perphenazine from perphenazine–amitriptyline tablets—automated fluorescence analysis in dissolution studies □ Dissolution rate testing of perphenazine–amitriptyline tablets—automated fluorescence analysis of perphenazine □ Fluorometry—analysis, automated, perphenazine from perphenazine–amitriptyline tablets during dissolution studies

A procedure based on the reaction between palladium (II) chloride and certain phenothiazines, including perphenazine, was reported previously (1, 2). This procedure was shown to be specific for perphenazine in the presence of certain oxidative degradates, specifically the sulfoxide. However, some interferences were encountered when applying this procedure to determinations of perphenazine. The interferences were due to excipients and could be avoided only by employing sample blanks. Furthermore, the palladium chloride assay was not sensitive enough to determine the low levels of perphenazine found in dissolution studies (0.5–5 μg/ml).

Mellinger and Keeler (3) described the identification of some phenothiazine drugs, including perphenazine, by monitoring the fluorescence obtained after reacting the drugs with potassium permanganate. This procedure has been automated and applied to the determination of perphenazine in dissolution studies of perphenazine–amitriptyline[1], a tranquilizer–antidepressant combination, in various proportions. Amitriptyline was removed by extraction prior to the permanganate reaction because it (or the oxidative products) interfered with the fluorescence of perphenazine. No sample blank was required.

EXPERIMENTAL

Materials—The following were used: chloroform, hydrochloric acid (0.1 *N*), and potassium permanganate solution (0.01% in 0.1 *N* HCl). Methanol and 0.1 *N* HCl served as purge solutions. All materials were reagent grade.

Apparatus—The analytical train consisted of the following: a liquid sampler II[2] and a proportioning pump[2], model I; a recording fluorescence spectrophotometer[3] equipped with a ratio mode and a flow cell (0.5-ml capacity and designed to measure fluorescence at an angle of 90° to the excitation beam); and a recorder[4].

[1] Triavil, Merck & Co.
[2] Technicon Corp., Tarrytown, N.Y.
[3] MPF-2A, Perkin-Elmer Corp., Norwalk, Conn.
[4] Sargent model SRL.

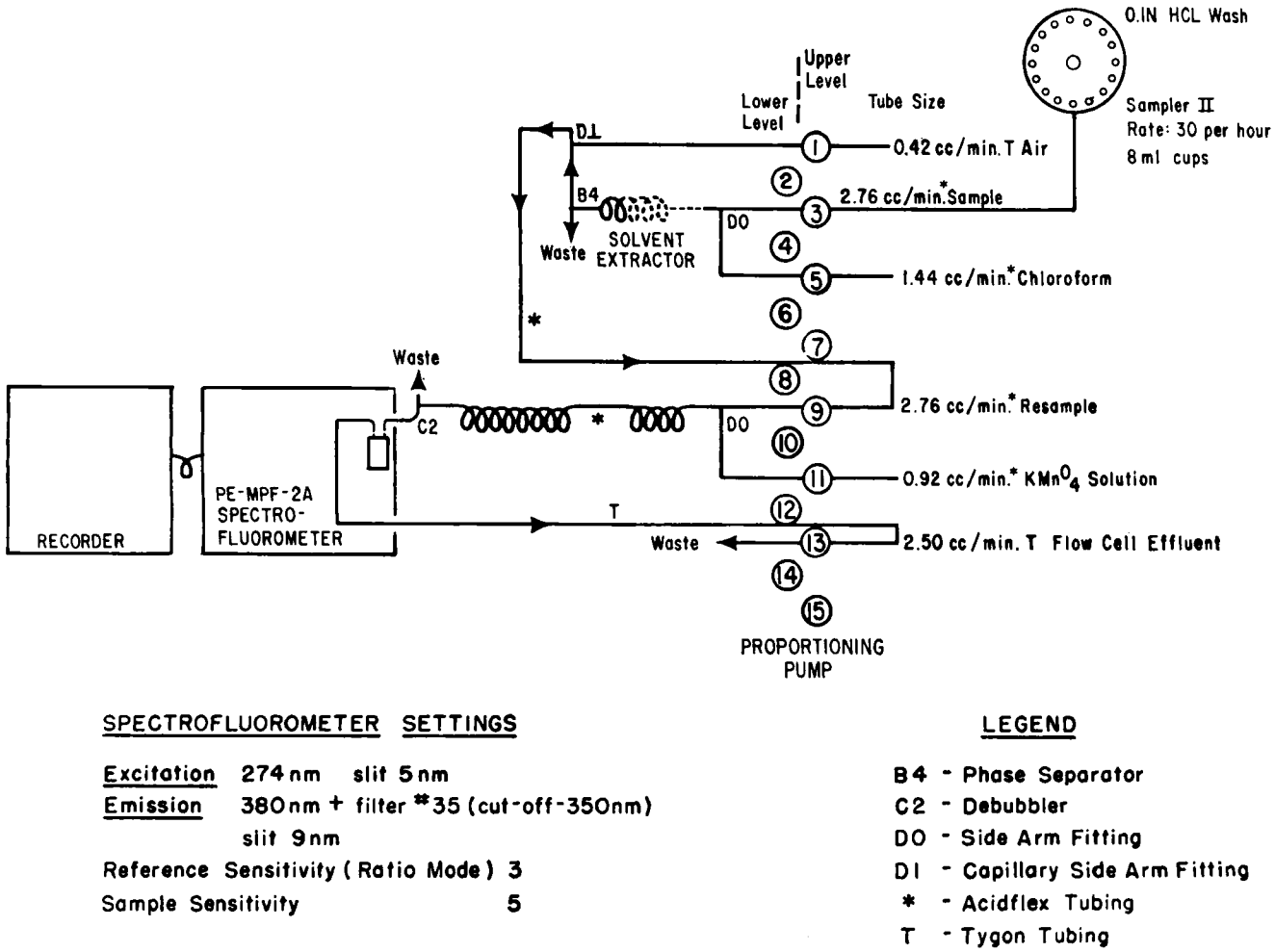

Figure 1—*Flow diagram for automated fluorescence perphenazine assay.*

Standard Preparation—Standards were prepared in 0.1 *N* HCl at concentrations of 1.2, 1.8, 2.4, and 3.0 μg/ml. For dissolution studies, this was equivalent to a sample concentration range of 45–110% of tablet claim. All standards were stored away from sunlight. Ordinary laboratory light was acceptable. Standards were prepared fresh daily.

Sample Preparation—In dissolution studies of tablets containing 2 mg of perphenazine, no dilution was required (sample concentration 0–2 mg/750 ml).

In dissolution studies involving tablets containing 4 mg of perphenazine, standard concentrations can be doubled to avoid sample dilution. (A lower instrument sensitivity can be employed.)

Approximately 5 ml of solution, sampled through a fritted gas dispersion tube, was transferred to the automatic sampler. Samples were stored away from sunlight.

Procedure—A schematic diagram of the automated system is shown in Fig. 1. All tubing was rinsed with methanol for 2 min and with 0.1 *N* HCl for 2 min prior to the introduction of reagents. The 0.1 *N* HCl was introduced first, followed by chloroform and potassium permanganate solution.

The most concentrated standard was introduced and the spectrofluorometer was adjusted as follows: excitation wavelength, a maximum near 274 nm; emission wavelength, a maximum near 380 nm; excitation slit, 5 nm; emission slit, 9 nm; emission cutoff filter, No. 35 (cuts out wavelengths below 350 nm); reference sensitivity, 2; and sample sensitivity, 5.

The sampling rate was 30/hr. The standard highest in concentration was included after every sixth sample to monitor possible

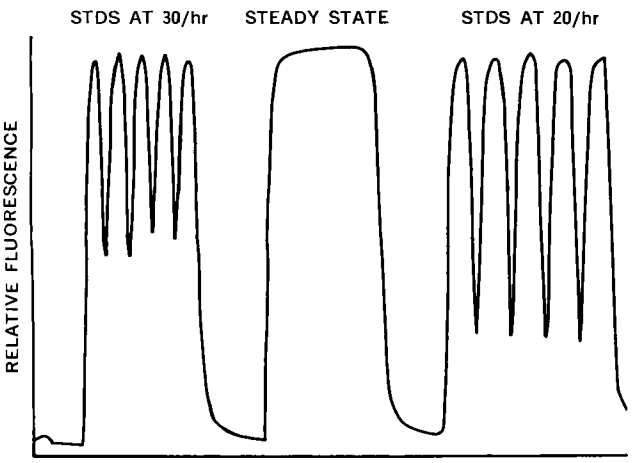

Figure 2—*Strip chart showing system response of standards at 3 μg/ml at rates of 20 and 30/hr compared to steady state.*

Table I—Correlation between Manual Palladium Chloride and Automated Fluorescence Methods

Lot	Palladium Chloride, % Claim	Automated Fluorescence, % Claim
1	104	102
2	101	101
3	108	107
4	99.5	98.0
5	94.5	94.5
6	95.5	94.5
7	96.0	98.0

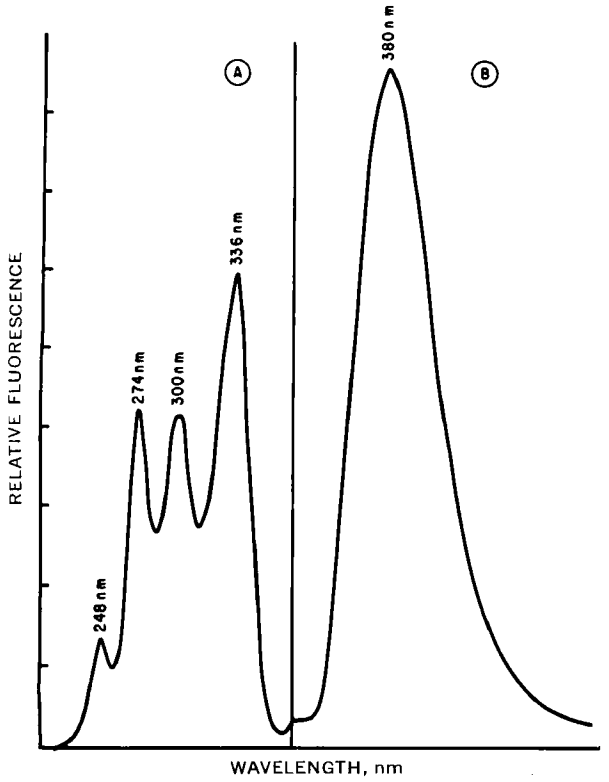

Figure 3—*Excitation (A) and emission (B) spectra for permanganate-oxidized perphenazine fluorophore.*

drift. Steady-state curves along with the flow response of standards sampled at rates of 20 and 30/hr are shown in Fig. 2.

DISCUSSION

The excitation spectrum of permanganate-oxidized perphenazine consists of four maxima of different intensities near 248, 274, 300, and 336 nm. The emission spectrum consists of one maximum near 380 nm. (See uncorrected spectra, Fig. 3.) Maximum sensitivity and reproducibility with minimum background interferences (<1%) from reagents and excipients were obtained when fluorescence was measured at an excitation wavelength near the maximum of 274 nm (emission at 380 nm). A filter fluorometer did not provide this resolution and lacked sufficient sensitivity. A fluorescence spectrophotometer[3] equipped with a xenon source was employed. The excitation slit was set at 5 nm for optimum resolution. The ratio mode incorporated in the amplifier of this equipment provided a constant output signal for several hours.

At an excitation wavelength of 274 nm and an emission wavelength of 380 nm, the fluorophore was stable for 3–10 min after addition of permanganate solution. The analytical train was arranged so that reaction time was 4 min. The fluorophore was not as stable at other excitation maxima.

Amitriptyline distorted the excitation spectrum, causing positive interference in some areas and quenching in others. This difficulty was avoided by extracting amitriptyline as an ion-pair from 0.1 *N* HCl using chloroform. In the analytical train, this operation was carried out before the addition of permanganate solution using an extraction coil and a phase separator, with the chloroform going to waste.

Ordinary laboratory light had no effect on fluorophore stability. However, solutions of perphenazine in 0.1 *N* HCl (20 μg/ml) exposed to sunlight for 2 hr on a windowsill exhibited a 15–20% loss when assayed by permanganate oxidation–fluorescence. Throughout the analysis, solutions of perphenazine and the analytical train were protected from sunlight.

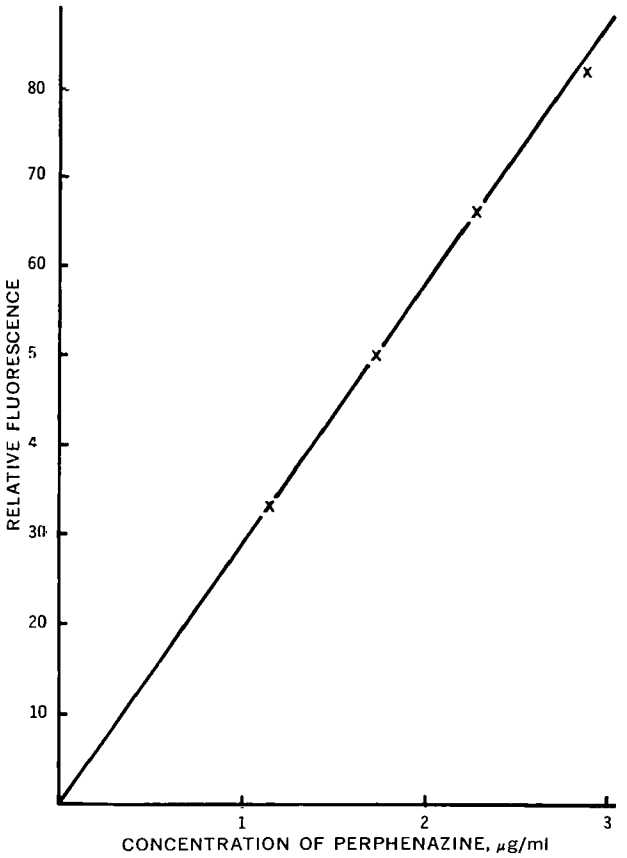

Figure 4—*Plot of fluorescence* versus *concentration of permanganate-oxidized perphenazine fluorophore.*

RESULTS

Following the described procedure, the relative fluorescence of perphenazine over a concentration range of 1.2–3.0 μg/ml was a straight line passing through the origin (Fig. 4). An identical tracing was obtained over the same concentration range for perphenazine plus a placebo containing all excipients including amitriptyline. Recovery from the placebo was 100% (at sampling rates of 20 and 30/hr), indicating that there was no positive interference and no quenching due to excipients.

Analyses of 32 standard solutions over a concentration range of 1.2–3.0 μg/ml plus all ingredients of a perphenazine–amitriptyline product[1] gave a relative standard deviation of ±1.7%. The samples were run at a 30/hr rate in staggered sequence. Sample peaks were 93–95% of steady state, depending upon the sampling rate (Fig. 2).

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