Determination of Erythromycin in Pharmaceutical Preparations by Direct Densitometry after TLC

C. RADECKA, W. L. WILSON[A], and D. W. HUGHES

Abstract □ An accurate, precise, and specific method for the determination of erythromycin base and estolate is described. The method is based on direct densitometric assay of colored spots formed with a spray reagent after resolution of the base and estolate by TLC.

Keyphrases □ Erythromycin and erythromycin estolate formulations—analysis, direct densitometry after TLC separation □ Densitometry, direct—analysis, erythromycins □ TLC—separation, erythromycins

The official assay method in Canada for the broad spectrum antibiotic erythromycin is microbiological (1, 2). Kuzel *et al.* (3) and Kuzel and Coffey (4) also described a spectrophotometric method which may be used either manually (3) or in automated systems (4). Neither of these methods differentiates among the various active components of the erythromycin complex, nor between erythromycin and those of its esters in current medical use. In the latter case, the esters are first hydrolyzed and the resulting erythromycin assayed. The BP and USP rely on separate identification tests for esters to differentiate these from erythromycin itself: paper chromatography in the former case (1) and IR spectroscopy in the latter (2).

Since the completion of this work, a GLC assay method for erythromycin has been described (5), in which volatile trimethylsilyl derivatives of constituents of the complex are resolved and then analyzed by integration of electrical responses from a flame-ionization detector.

The direct quantitation of compounds separated on TLC plates has been applied successfully to the analysis of the tetracycline antibiotics (6), with a marked improvement over official microbiological procedures. This paper describes a rapid and simple method for the simultaneous and specific identification and assay of either erythromycin estolate (the most commonly used ester of erythromycin) or erythromycin base. It relies on initital TLC resolution of the base and the estolate, followed by direct densitometric assay of colored spots formed with a spray reagent. The results are compared with those obtained using official microbiological techniques for seven pharmaceutical formulations.

EXPERIMENTAL

Standard Solutions—Erythromycin base USP and estolate NF reference standards were dissolved and made up to appropriate concentrations in methanol (approximately 5 and 10 mg./ml., respectively).

Assay Solutions—Twenty capsules of erythromycin formulation were selected at random, weighed, and emptied, and the weight of the contents was obtained by difference. An accurately weighed sample of powder, approximately equivalent to the contents of an average capsule, was dissolved in methanol to give a concentration from the label claim of about 5 mg./ml., calculated as erythromycin base. In the case of capsules containing excipients insoluble in methanol, the suspensions were centrifuged prior to use and the supernatant solutions were used for subsequent spotting. Samples of bulk powder or powder formulated to be made up into aqueous suspensions were weighed and dissolved directly.

TLC—TLC plates (20 × 20 cm., 0.25 mm. thickness) were prepared with standard equipment using a slurry of commercial silica gel G[1] (50 g.) with 0.02 *N* aqueous sodium acetate (100 ml.). The plates were air dried overnight. The solvent system was methanol–0.02 *N* aqueous sodium acetate (130:20), and the chromatographic chamber, lined with filter paper, was saturated with the solvent vapor for 1 hr. prior to use. Hamilton syringes, attached to a repeating dispenser, were used for application of samples to the plates.

Densitometric Assay—Three identical aliquots of the sample to be analyzed were applied to the starting line in the central region of the plate at a distance of 2 cm. above the lower edge. Four different concentrations of reference sample were applied as spots in increasing concentration from left to right across the plate, two spots being on each side of the three unknowns (Fig. 1). The plate was developed to about 15 cm. (40 min.), removed from the tank, uniformly sprayed with a solution of glucose (2 g.) in a mixture of 85% phosphoric acid (10 ml.), water (40 ml.), ethanol (30 ml.), and *n*-butanol (30 ml.) (7), and then heated for 5 min. at 150°. The resulting gray-brown spots were analyzed within 1 hr. by direct densitometry[2].

The colored spots were scanned in the direction of solvent flow. The aperture (0.1 mm. width) was adjusted so that it was just long enough to encompass the largest spot in any given run. The searchhead was adjusted to about 2 mm. above the upper surface of the chromatographic plate. All readings were taken in a darkened room.

Standard curves were prepared for each individual plate by plotting the integrals of the observed peaks (integrator readings) for the four spots of reference standard *versus* the amounts (micrograms) spotted on the plate (Fig. 2). The values for the unknown sample, applied in triplicate and prepared in such a manner that the ap-

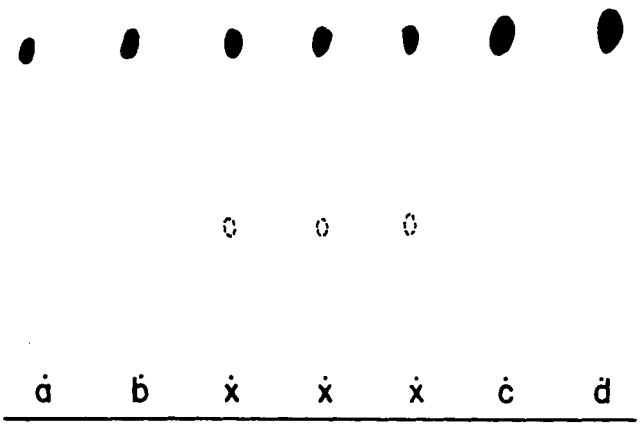

Figure 1—*Representative chromatogram for densitometry. Key: a, 1 μl. erythromycin estolate standard; b, 2 μl.; x, unknown concentration of erythromycin estolate sample; c, 3 μl.; and d, 4 μl.; lower spot indicates position of erythromycin base.*

[1] Merck.

[2] A densitometer (model 520M, Photovolt Corp., New York, N. Y.) equipped with a motor-driven TLC stage [5.08 cm. (2.0 in.)/min.], a Varicord 42B recorder [5.08 cm.(2.0 in.)/min.], a search unit C, and an Integraph integrator (model 49) was employed.

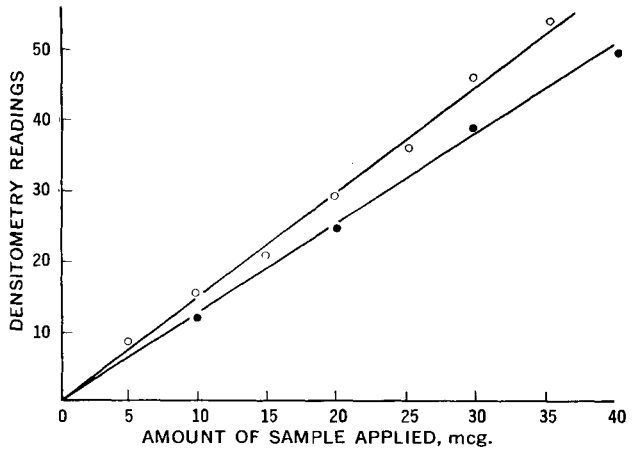

Figure 2—*Representative standard curves for erythromycin base and estolate. Key: ○, erythromycin base; and ●, erythromycin estolate.*

proximate concentration (label claim) was between the values of reference standards, were found from the curve. The results were expressed as a percentage of label claim.

RESULTS AND DISCUSSION

A number of published and novel TLC systems and sprays were tried in this work before deciding that the ones chosen (see *Experimental*) gave, in this study, optimum results for densitometric assay. Under the conditions described, a linear relationship between integrated densitometric readings and amount of material applied was established over the range 5–30 mcg. (Fig. 2).

Results of the determination of erythromycin by this densitometric method and by microbiological assay are presented in Table I. Generally, there is good agreement between the two procedures; in addition, the results obtained by densitometry are more precise than the officially required precision (±5%) of the microbiological assay (1). The method has the added advantage of rapidity, especially since long incubation times are avoided.

The low densitometric results for the erythromycin estolate capsules (Samples C and D) are within the allowed error of the two methods but may also be accounted for by slight hydrolytic decomposition in the samples, since the densitometric comparison was made directly with an NF sample of estolate, the microbiological assay being referred to erythromycin base. This specificity is perhaps the chief advantage that the method has over the official method, in which samples of estolate are first hydrolyzed to the base which is then assayed microbiologically, the results being customarily expressed on the label in terms of equivalence to erythromycin base. Thus, aged or degraded formulations could give satisfactory assay results, even though containing little or none of the unhydrolyzed base. In many instances, samples of erythromycin esters have produced traces of spots on TLC corresponding to erythromycin base. Tsuji and Robertson (5) also determined the presence of 6.2% erythromycin base in a sample of erythromycin estolate using TLC and GLC.

The spectrophotometric method (3, 4) shares with the official method the disadvantage of a lack of specificity, but it is rapid, simple, and capable of being adapted as an automated method. The recently described GLC method of Tsuji and Robertson (5) appears to offer better resolution of the erythromycin complex and ester derivatives than other chromatographic systems and also greater accuracy than this densitometric method. However, under the conditions described, erythromycin B has a similar retention time to erythromycin estolate and might interfere with the quantitation. The GLC assay also involves a lengthy (24 hr.) chemical manipulation of the compounds prior to resolution and the use of somewhat extreme temperature conditions. This latter point may account for difficulties that the authors and others (8) encountered in attempting assays of similarly high molecular weight compounds by GLC of trimethylsilyl derivatives. The two techniques (TLC/densitometry and GLC) are to a great extent complementary, depending on the requirements of the analysis. The densitometric method lacks the extremely high accuracy and resolving power of the GLC method, but it does have advantages of simplicity and easy reproducibility together with sufficient resolution and accuracy for current pharmacopeial requirements.

Potential problems due to errors in spot application, layer nonuniformity, and unevenness of spraying were discussed elsewhere (6, 9) and are satisfactorily overcome if standards and unknowns are compared under identical conditions on the same plate as described here.

Table I—Results of Microbiological and Densitometric Assays of Erythromycin

Sample	Dosage Form	Densitometric Assay[a] Erythromycin Base Found, %	Densitometric Assay[a] Erythromycin Estolate Found, %	Microbiological Assay, Erythromycin Base Found, %
A	Erythromycin base, bulk	94.8 ± 3.4[b]	—	94.0[c]
B	Erythromycin base, bulk	96.9 ± 2.1	—	97.5
C	Erythromycin estolate, capsules	—	97.1 ± 2.7	103.0
D	Erythromycin estolate, capsules	—	95.0 ± 3.4	96.0
E	Erythromycin estolate, bulk	—	95.6 ± 3.2	90.7
F	Erythromycin estolate, bulk	—	89.1 ± 2.6	89.9
G	Erythromycin estolate, powder (for suspension)	—	98.4 ± 2.5	96.0

[a] Average of a minimum of seven determinations for each value reported. [b] Standard deviation. [c] Determinations made by Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada, with required pharmacopeial precision of ±5%.

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▲ To whom inquiries should be directed.