Note

Quantitative determination of potassium penicillin G by thin-layer spectrodensitometry

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(Received April 9th, 1973)

Recent publications have cited the usefulness of thin-layer spectrodensito-metry (TLS) for the determination of a variety of pharmaceutically interesting com-pounds. Among the compounds quantitated by this technique were quinine sulfate[1] steroids[1–3], methyl and propyl parabens[4], barbiturates[5], the antibiotics tetracycline[6] and erythromycin[7], as well as ergometrine, ergotamine and ergocristine[8]. Because of the concurrent use of a separation and quantitation technique, analysis by TLS offers specificity. Sensitivities comparable to other optical methods of analysis can be achieved by using the inherent adsorption or emission properties of the analyte or by forming suitable derivatives.

The most popular methods for quantitating intact penicillins have made use of hydroxylamine, iodometric titration, or microbiological procedures[9]. The appli-cation of spectroscopic techniques has been limited by the structural similarity of penicillins and the similarity of a particular penicillin to its degradation products. Paper and thin-layer chromatography (TLC)[10–13] have been used for the qualitative analysis of penicillins as has gas chromatography[14]. TLS appeared to be a useful alternative for the analysis of penicillins. The purpose of this investigation was to develop a quantitative procedure that could be applied to the analysis of potassium penicillin G in pharmaceutical preparations. The information gained by studying this prototype should be applicable to structurally related compounds.

EXPERIMENTAL

Materials and methods

Commercial penicillins. Potassium penicillin G was obtained from Pfizer,

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New York, N.Y., and was used without further purification. Buffered potassium penicillin G for injection USP XVIII (5 million units) and Pentids for syrup (125 mg/5 ml and 250 mg/5 ml) were manufactured by Squibb, New York, N.Y. Sugracillin 250M was manufactured by Upjohn, Kalamazoo, Mich.

Thin-layer chromatography. Silica gel plates (E. M. Labs., Elmsford, N.Y.) (20×20 cm) were scored into 1-cm lanes with an SDA-320 TLC plate scorer (Schoeffel, Westwood, N.J.). Alternate lanes were spotted, permitting the use of a reference lane for each sample lane. Standard solutions of potassium penicillin G in doubly-distilled water were prepared at concentrations of 1 and 10 μg/μl. Potassium penicillin G dosage forms were reconstituted as instructed by the manufacturer using doubly-distilled water and appropriate dilutions were made prior to spotting. Appropriate volumes of the standard solutions and the potassium penicillin G dosage forms were applied with a 10-μl Hamilton syringe, each application being dried in a nitrogen stream before another was made. Chromatograms were developed in 7×28×22 cm glass tanks lined with Whatman No. 1 filter-paper. The solvent system used was acetone–chloroform–glacial acetic acid (50:45:5) (ref. 13). All plates were developed until the solvent rose to within 5 cm of the top (*ca.* 50 min).

Densitometry. A Schoeffel Model SD-3000 double-beam spectrodensitometer equipped with a high-pressure mercury–xenon lamp was used. A quartz prism monochromator permitted the incident radiation to be varied from 200 to 700 nm. All measurements were made at 230 nm in the reflectance mode at an attenuation output of 0.2 absorbance units full scale; this analytical wavelength was employed in order to maximize sensitivity. A Schoeffel SDR 303 recorder was used. Peak areas were determined by multiplying peak height by the peak width at half-height.

Iodometric assay. Potassium penicillin G dosage forms were assayed for intact penicillin by a modified iodometric titration procedure[15]. To 2.0-ml aliquots containing 1–3 mg of potassium penicillin G, 2.0 ml of 1N sodium hydroxide were added and samples were allowed to stand at room temperature for 15 min. At the end of this time, 2.0 ml of 1.2 N hydrochloric acid were added followed by 10 ml of 0.01 N iodine. After 15 min, the excess iodine was titrated using 0.01 N sodium thiosulfate. For the blank determinations, a 2.0-ml sample was titrated immediately after the addition of 10 ml of 0.01 N iodine.

RESULTS AND CONCLUSIONS

The relationship between the amount of sample spotted and the precision of measuring the peak area was determined on two different plates. As expected, more precise values were obtained from larger samples. A standard deviation of approx. 5% was calculated for zones containing 1 μg of potassium penicillin G while 10-μg zones showed a value of *ca.* 1.5%.

Fig. 1 demonstrates the relationship between peak area and concentration; linearity is noted from 1–20 μg. This was confirmed by computing the correlation coefficients of the least squares regression lines for various concentration ranges applied to six different TLC plates. In each case the correlation coefficient was approx. 1. However, the slope and intercept of the regression line varied significantly from plate-to-plate. Routinely, three standard lanes were interspersed among the nine lanes used for analysis.
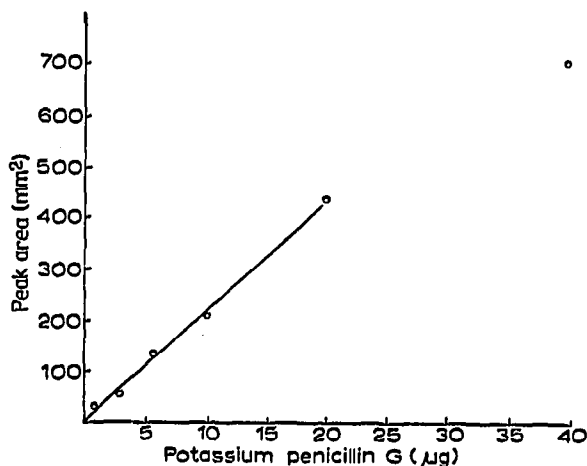
Fig. 1. Relationship between peak area and concentration for potassium penicillin G solutions.

The linear response cited above was not obtained if the zones were small and dense. When peak areas were measured immediately after spotting, but before development, linearity was seen only up to 6 μg. However, after development, linearity (correlation coefficient 0.999) was again observed up to 20 μg. These observations support the recommendation of Touchstone et al.[1] that R_F values of 0.25–0.75 are desirable to avoid either highly concentrated zones or excessively dispersed spots that could become contaminated by the solvent front.

Because penicillin solutions are unstable the possible decomposition of potassium penicillin G during the analysis was considered. Peak areas were compared before and after development. From 1–6 μg, the range in which peak area measured before development was found to vary linearly with the amount spotted, no statistically significant difference between peak areas measured before and after development was obtained, indicating that losses of penicillin G during the time of quantitation were not extensive.

TABLE I

ASSAY OF COMMERCIAL POTASSIUM PENICILLIN G DOSAGE FORMS

Dosage form	TLS		Iodometric	
	μg spotted*	μg found (% theory)	mg sample*	mg found (% theory)
Pentids for syrup, 125/mg/5 ml	6.25	6.6 (106)	2.0	2.12 (106)
	12.5	13.9 (111)		
Pentids for syrup, 250 mg/5 ml	6.25	6.7 (107)	2.0	2.17 (108)
	12.5	13.8 (110)		
Sugracillin 250M	7.5	8.3 (110)	1.2	1.38 (115)
	15.0	16.4 (109)		
Buffered potassium penicillin G for injection USP	12.5	13.1 (105)	2.5	2.61 (105)

* Sample based on labelled potency.

The amount of decomposition of potassium penicillin G during analysis was approximated by studying the UV spectrum of the sample after development. The spectrum of sample isolated from the chromatoplate by extraction with absolute ethanol exhibited the same spectral features as a freshly prepared solution of potassium penicillin G except that an additional maximum was noted at 322 nm. The maximum at 322 nm was also present when the UV spectrum of the potassium penicillin G spot was measured from a developed chromatoplate without removing the sample from the adsorbent. This maximum is characteristic of penicillenic acid which possesses an ε value of 30,600 at 322 nm (ref. 16). From these data it was calculated that 2% conversion to penicillenic acid could have occurred during quantitation.

To illustrate the application of TLS to pharmaceutical dosage forms, three commercial reconstituted syrups and one injectable solution were assayed. For comparison purpose the standard iodometric determination of potassium penicillin G was performed. As seen in Table I the results obtained by the two methods compared favorably and reflect the normal overage which most penicillin dosage forms contain. Significantly, TLS yields comparable data from samples that are several hundred times smaller than those required by the iodometric technique.

ACKNOWLEDGEMENT

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