Rapid screening of marketed paracetamol tablets: use of thin-layer chromatography and a semiquantitative spot test

J. Roy,¹ P. Saha,² S. Sultana,³ & A.S. Kenyon⁴

Evaluated is the use of thin-layer chromatography (TLC) to determine the quality of paracetamol tablets marketed in Bangladesh. The procedure was carried out using a cheap and rapid TLC method developed by the U.S. Food and Drug Administration. Reported also is a semiquantitative specific spot test for screening paracetamol tablets. The results obtained indicate that, of the 38 brands tested, three were spurious, while 11 were of borderline quality. In some cases, the results were also verified using the British Pharmacopoeia method.

The simplified tests described in this article cannot replace the British Pharmacopoeia or U.S. Pharmacopoeia methods but can be employed as initial screening tests.

Introduction

The quality of marketed drugs determines the quality of treatment patients receive, which in turn ensures their well-being. On the other hand, a patient’s health can be put at risk by the use of spurious and substandard drugs. Constant screening of marketed drugs by the drug regulatory authority or a consumer organization, using pharmacopoeial methods, therefore enables consumers to be aware of the quality of drugs available to them. However, pharmacopoeial methods are not straightforward or inexpensive to carry out in most developing countries, and numerous small and medium-sized pharmaceutical companies do not analyse their drugs before they are marketed because of the considerable expense of maintaining a proper quality control laboratory.

The Division of Drug Analysis, U.S. Food and Drug Administration (FDA), has therefore developed a simple and inexpensive semiquantitative method (1–3) for rapidly screening pharmaceuticals using thin-layer chromatography (TLC). This method has been employed successfully in Swaziland, Egypt, and Saudi Arabia (3–5). Use of this TLC method to monitor the quality of paracetamol tablets currently available in Bangladesh would be desirable, since almost a third of such tablets have been reported to be spurious (6).

The study had the following aims: to determine the applicability of the TLC method for rapidly screening paracetamol tablets; and to develop a cheap, semiquantitative, specific spot test for paracetamol tablets. The results we report here are preliminary and indicate that further studies should be carried out.

Materials and methods

Paracetamol tablets

Various brands of paracetamol tablets were bought from both city and village retail pharmacy shops in Bangladesh. Reference tablets of paracetamol in sealed strips were supplied by FDA.

Thin-layer chromatography

Equipment and chemicals. The TLC apparatus consisted of the following items:

- plastic-backed TLC sheet of silica gel 60, F-254 (E. Merck, Darmstadt, Germany);
- aluminium TLC support tray;
- rigid aluminium support;
- polyethylene plastic development bag;
- heavy filter-paper saturation pad;
- small plastic bag for crushing paracetamol tablets;

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— small S-hook positioning rod;
— disposable micropipettes (3 µl) with small rubber bulbs; and
— ultraviolet (UV) TLC scanner for visual analysis.

The following chemicals were used:
— paracetamol reference tablets;
— methanol; and
— ethyl acetate and concentrated ammonium hydroxide solution.

The British Pharmacopoeia (BP) method (7) for the assay of paracetamol was used to check the results of the TLC. The method is based upon titration with 0.1 mol/l ammonium cerium(IV) sulfate after the sample has been dissolved in acid and the mixture refluxed. Iron(II) sulfate solution (0.1 mol/l) is used as the indicator.

**Procedure.** All solutions were prepared as described previously (4). Each marketed sample of paracetamol (500-mg tablets) was dissolved in methanol to give a solution of concentration 5 g/l; this represented the 100% sample solution. One reference tablet of paracetamol (46 mg) was dissolved in methanol to give a solution of concentration 5.75 g/l; this represented the high concentration (115%) reference solution. An aliquot of the 115% solution was then diluted to give the low concentration (85%) reference solution (a spread of 85–115% in acceptable for single tablet analysis; however, for a sample prepared from an aliquot of 20 ground paracetamol tablets, the limits are 90–110%). A 3-µl aliquot of each of the sample and reference solutions was spotted onto the TLC plate — 85% solution on the left, the sample (100%) in the centre, and the 115% solution on the right. The spotted plate was developed using an eluent consisting of a mixture of methanol, ethyl acetate, and concentrated ammonium hydroxide (2:17:1). After the plate was dried, the spots \( R_f = 0.45 \) were viewed using a UV TLC scanner at \( \lambda = 254 \text{ nm} \).

**Spot test**

The spot test apparatus consisted of the following items:
— heavy filter-paper; and
— disposable micropipettes (3 µl) with small rubber bulbs.

The following chemicals were used:
— paracetamol reference tablets;
— iron(III) chloride solution (0.5 mol/l); and
— methanol.

**Results and discussion**

**Quality of paracetamol tablets marketed in Bangladesh**

A total of 38 brands of paracetamol tablets (paracetamol capsules are not available in Bangladesh) were screened for their content of the active ingredient. A single tablet was used when the quality of the brand was assessed using the rapid TLC method. Several runs were carried out for each tablet and the combined results of three workers were recorded. In some cases where the results were conflicting, sampling was repeated. Of the 38 brands tested, 23 (60.5%) were compliant and only 3 (8%) were spurious, far below the 30% reported previously (6). One reason for this improvement is that the licences of several small, substandard pharmaceutical companies have been revoked over the past 2 years.

**Use of the TLC method and its validation**

Because only one tablet of paracetamol was used in each analysis, the U.S. Pharmacopoeia (USP) range of ±15% for individual drug tablets was taken as the standard. However, when the content of active ingredient was calculated based on the average weight of 20 tablets, the BP and USP ranges for 500-mg paracetamol tablets are only ±5% and ±10%, respectively. We found that 11 brands were close to the lower range limit of 85% and that three brands were far below this. There was no difficulty in identifying these three brands when the intensity and size of the developed spots were compared with those of the reference samples. However, there was some confusion when the intensity of the spots approached that of the lower limit; these brands were termed borderline and were also analysed using the official BP method. The results are compared in Table 1.

To maximize the difference in intensity between the two limits, we compared spots using serial dilutions of the reference solution. A concentration of 0.125–0.5 g/l was found to be the optimum for the semiquantitative analysis. In addition, a standard curve was obtained (Fig. 1) based on the area of the reference spots, calculated using the
Table 1: Comparison of the quantitative estimation of paracetamol using the TLC and British Pharmacopoeia (BP) methods

<table>
<thead>
<tr>
<th>Sample code</th>
<th>TLC method: &lt;85% limit</th>
<th>Close to 85% limit&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BP method (potency, mg/tablet)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>3</td>
<td>—</td>
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</tr>
<tr>
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<tr>
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<sup>a</sup> Borderline.
<sup>b</sup> BP limits: 500 ± 5% mg/tablet.

Few marketed samples of paracetamol tablets have been analysed for their paracetamol content relative to the standard at low concentration. The results obtained were verified by comparing them with those obtained using the BP method (Table 2).

**Specific spot test**

For routine analysis, TLC plates, such as the silica gel 60 F-254 sheets used in the present study, are expensive; also, the developing solvents are hazardous to some extent. We therefore investigated a qualitative test for paracetamol using iron(III) chloride indicator, adapted for semiquantitative purposes. Four solutions of iron(III) chloride of different concentration (5%, 10%, 15%, and 20%) were prepared and small pieces of heavy duty filter-paper were impregnated with them and dried. Reference standard solutions of paracetamol (85% and 115%) were then spotted on to the iron(III)-chloride-treated filter-paper. A distinctive difference in colour intensity was produced in each case, with the optimum results being obtained with the 10% solution of iron(III) chloride (Fig. 2). The optimum

**Fig. 2. Appearance of the spot test results for paracetamol analysis.** (L = 85% of reference; D = drug being analysed; R = 115% of reference). (A = spurious sample; B = sample within range; C = sample close to lower limit).

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Table 2: Paracetamol content of tablets obtained using the standard TLC curve and the British Pharmacopoeia (BP) method

<table>
<thead>
<tr>
<th>Sample code</th>
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<th>BP method</th>
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**Fig. 1. Standard calibration curve of spot area versus concentration for the TLC analysis.**
concentration of paracetamol for the estimation was found to be approximately 5 g/l. The brands of paracetamol marketed in Bangladesh were then analysed by interpolation between the upper and lower limits.

Acknowledgements
We gratefully acknowledge the gift of the TLC kits from the Division of Drug Analysis, U.S. Food and Drug Administration. The financial support of the U.S. Agency for International Development for the manufacture of the TLC kits and reference tablets of paracetamol is gratefully acknowledged.

Résumé
Contrôle rapide des comprimés de paracétamol vendus dans le commerce: utilisation de la chromatographie en couche mince et d’un test semi-quantitatif sur papier filtre
Cette étude évalue l’utilisation de la chromatographie en couche mince (TLC) pour déterminer la qualité des comprimés de paracétamol commercialisés au Bangladesh. La technique utilisée était une technique rapide et peu coûteuse de TLC développée par l’US Food and Drug Administration. Un test semi-quantitatif sur papier filtre est également décrit.
Les résultats obtenus indiquent que sur les 38 spécialités testées, trois étaient frelatées et 11 étaient de qualité limite. Dans certains cas, les résultats ont été vérifiés selon la méthode de la pharmacopée britannique.

Les tests simplifiés décrits dans le présent article ne peuvent remplacer les méthodes de la pharmacopée britannique ou de la pharmacopée des États-Unis d’Amérique, mais peuvent être employés à titre de contrôle initial.

References