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A limit test for *p*-chloroacetanilide and other impurities in paracetamol and phenacetin using thin-layer chromatography

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A thin-layer chromatography procedure is described suitable as a limit test for *p*-chloroacetanilide in paracetamol and phenacetin. The sample is chromatographed on silica gel together with a standard using the solvent mixture cyclohexane: acetone: diisobutylketone: methanol: water (100:80:30:5:1) and detection is by irradiation with ultraviolet light 253·7 mμ followed by examination in light of wavelength 365 mμ. The procedure may be used to limit the *p*-chloroacetanilide content of tablets containing paracetamol or phenacetin. Other possible impurities in paracetamol and phenacetin are also detected and may be limited by similar procedures.

THE undesirable side-effects of phenacetin may be due to phenacetin itself or to impurities such as *p*-chloroacetanilide. Harvald, Valdorf-Hansen & Nielsen (1960) have suggested that renal damage arises from the methaemoglobin-forming activity of *p*-chloroacetanilide rather than from any direct effect of phenacetin or related drugs.

Methods for the determination of *p*-chloroacetanilide in phenacetin include Raney nickel reduction (Hald, 1951), a polarographic procedure (Jones & Page, 1964), paper chromatography (Ritter, Mutter & Hoffstetter 1961) and a spectrophotometric procedure (Crummett, Simek & Stenger, 1964). The content of *p*-chloroacetanilide in phenacetin is limited as follows: $\not>$ 0·11% (British Pharmacopoeia: Raney nickel reduction); $\not>$ 0·3% (Pharmacopoeia Nordica: Raney nickel reduction); $\not>$ 0·03% (United States Pharmacopeia: paper chromatography). The content of *p*-chloroacetanilide in paracetamol is limited to $\not>$ 0·11% (British Pharmacopoeia: Raney nickel reduction).

In our hands the paper chromatograms obtained by the U.S.P. method proved difficult to interpret because of the diffuse nature of the *p*-chloroacetanilide spots. The thin-layer chromatography procedure described below is more rapid, more sensitive and more easily interpreted.

Experimental

The following procedures are designed to limit the *p*-chloroacetanilide content of paracetamol or phenacetin or tablets containing these substances to not more than 0·03% of the drug.

Apparatus and reagents. Microcap pipettes, 2 and 5 μl capacity. Sources of ultraviolet light of 253·7 mμ and 365 mμ.* Kieselgel G (Merck): 0·25 mm layer spread on 20 × 20-cm glass plates using standard techniques; activated at 110° for 1 hr; stored over anhydrous silica gel.

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* A 15 W germicidal lamp (Mazda) was used for the former and a 125 W mercury vapour lamp for the latter.

Solvent mixture: analytical grades of cyclohexane, acetone, diisobutylketone, methanol and water (100:80:30:5:1).

Paracetamol and phenacetin free from p-chloroacetanilide may be obtained by repeated recrystallisation of the compounds. Alternatively, they may be conveniently obtained from manufacturers who do not use chlorinated intermediates in their preparation. Freedom from *p*-chloroacetanilide may be established either by the spectrophotometric method (Crummett & others, 1964; limit of detection, 10 ppm) or by the thin-layer procedure using a 900 μg loading on a 0·5 mm layer of Kieselgel G, running the chromatogram 7·5 cm with diisobutylketone as solvent and irradiating for 15 min at 253·7 mμ (limit of detection: 30 ppm).

I. PROCEDURE FOR PARACETAMOL AND PHENACETIN

Standard Solution A: Dissolve *p*-chloroacetanilide-free phenacetin (300 mg) in dichloromethane (8 ml), add 1 ml of 0·009% w/v solution of *p*-chloroacetanilide in dichloromethane and dilute to 10 ml with dichloromethane.

Standard Solution B: Dissolve *p*-chloroacetanilide-free paracetamol (1·50 g) in methanol (8 ml), add 1 ml of 0·045% w/v solution of *p*-chloroacetanilide in methanol and dilute to 10 ml with methanol.

Sample Solution A: Dissolve phenacetin sample (300 mg) in dichloromethane (8 ml) and dilute to 10 ml with dichloromethane.

Sample Solution B: Dissolve paracetamol sample (1·50 g) in methanol (8 ml) and dilute to 10 ml with methanol.

Method. According to whether phenacetin or paracetamol is being examined, apply 2 × 5 μl portions of Sample Solution A or 1 × 2 μl of Sample Solution B as a single spot about 2·5 cm from one edge of the chromatoplate. On the same spotting line apply, as appropriate, 2 × 5 μl portions of Standard Solution A or 1 × 2 μl of Standard Solution B as a single spot. The spots should be not less than 1·5 cm apart. Place the plate in the tank and allow the solvent to rise 15 cm past the spotting line. Dry the plate in a stream of cold air (10 min), hold it within 2–3 cm of the source of ultraviolet light (253·7 mμ) for 10 min and then examine under ultraviolet light (365 mμ). (Optimum irradiating conditions must be determined for the particular source used.) The intensity of fluorescence of the spot due to *p*-chloroacetanilide in the standard is compared with that of the spot with the same running distance in the sample.

Up to 6 samples of phenacetin or paracetamol can be examined on one plate, loading sample and standard solutions alternately. The total working time for the examination of 6 samples is about 90 min.

None of the undernoted substances interfere with the separation of *p*-chloroacetanilide from phenacetin or paracetamol or with its subsequent detection: maize starch, lactose, sucrose, acacia, alginic acid, gelatin, stearic acid, magnesium stearate, calcium stearate, talc, polyvinylpyrrolidone, sodium benzoate, acetylsalicylic acid, caffeine and codeine phosphate.

II. PROCEDURE FOR TABLETS OF PHENACETIN AND TABLETS OF PARACETAMOL

Use procedure I, replacing Sample Solutions A and B by the following:

Sample Solution C: To powdered tablet equivalent to 300 mg phenacetin in a 10 ml centrifuge tube, add sufficient dichloromethane to give a volume of 10 ml. Shake for 20 min, adjust to 10 ml with dichloromethane if necessary and centrifuge (5 min) at 1,000 rpm. Apply 2 × 5 μl portions of the supernatant liquid to the chromatoplate.

Sample Solution D: Repeat the extraction for Sample Solution C using powdered tablet equivalent to 1·50 g paracetamol and replacing dichloromethane by methanol. Apply a 2 μl portion of the supernatant liquid to the chromatoplate.

III. PROCEDURE FOR COMPOUND TABLETS OF CODEINE

Use procedure I, replacing Sample Solution A by Sample Solution C and Standard Solution A by Standard Solution D: Dissolve *p*-chloroacetanilide-free phenacetin (300 mg) and acetylsalicylic acid (300 mg) in dichloromethane (8 ml), add 1 ml 0·009% w/v solution of *p*-chloroacetanilide in dichloromethane and dilute to 10 ml with dichloromethane.

IV. PROCEDURE FOR COMPOUND TABLETS OF ACETYLSALICYLIC ACID

Use procedure I replacing Sample Solution A by Sample Solution C and Standard Solution A by Standard Solution E: Dissolve *p*-chloroacetanilide-free phenacetin (300 mg), acetylsalicylic acid (500 mg) and caffeine (100 mg) in dichloromethane (8 ml), add 1 ml 0·009% w/v solution of *p*-chloroacetanilide in dichloromethane and dilute to 10 ml with dichloromethane.

Standard solutions must contain the same amount of phenacetin and paracetamol as the sample solutions or false evaluations will result. Since acetylsalicylic acid and caffeine are soluble in dichloromethane and affect spot sizes and running distances, it is considered necessary to include them in the standards used for examining the compound tablets.

Under the conditions I to IV above, *p*-chloroacetanilide gives a bluish-white fluorescent spot which can be detected down to a level of 0·01%. There is a reasonably good gradation in intensity for standards containing 0·01, 0·02, 0·03, 0·04 and 0·05% of *p*-chloroacetanilide in paracetamol or phenacetin and an unknown in this range can be placed to the nearest 0·01%.

Results and discussion

Under the condition of Procedure I, *p*-chloroacetanilide runs approximately 8 cm and is separated from phenacetin and paracetamol and other impurities. The approximate running distances of some relevant compounds, relative to *p*-chloroacetanilide, are given in Table 1.

Samples of pharmaceutical-quality paracetamol and phenacetin and tablets containing these materials were examined by the recommended procedures and the results are given in Tables 2 and 3. The samples of paracetamol and paracetamol tablets contained no detectable *p*-chloroacetanilide, nor did three of the phenacetin samples, two of which were

probably manufactured by routes that do not involve chlorinated intermediates. With one exception, the tablets containing phenacetin did not contain more than 0·06% of *p*-chloroacetanilide. The exception contained 0·7%, a result which was confirmed by the spectrophotometric method (Crummett & others, 1964) and the Raney nickel reduction procedure (Hald, 1951).

TABLE 1. SEPARATION OF PHENACETIN, PARACETAMOL AND ASSOCIATED IMPURITIES UNDER CONDITIONS GIVEN IN PROCEDURE I

Compound	Running distance relative to *p*-chloroacetanilide	Approx. limit of detection as percentage in phenacetin or paracetamol
p-Nitrophenetole	1·50	†
Diacetyl-4-phenetidine	1·20	0·02
p-Nitrophenol	1·09	†
p-Phenetidine	1·07	0·04
p-Chloroacetanilide	1·00	0·01
Acetanilide	0·89	0·03
O-Acetylparacetamol	0·77	0·02
p-Aminophenol	0·70	0·01
Phenacetin	0·84 (0·73–0·95*)	
Paracetamol	0·54 (0·41–0·66*)	0·02

* Measured from rear to front of spots at 300 μg loading.
† These compounds do not give the bluish-white fluorescence which is given by the other compounds.

To check the recovery of *p*-chloroacetanilide, laboratory batches of tablets of phenacetin B.P.C. and compound tablets of acetylsalicylic acid B.P.C. were prepared from phenacetin containing 0·05% *p*-chloroacetanilide and were examined as described above. There was no apparent loss of *p*-chloroacetanilide.

TABLE 2. SAMPLES OF PARACETAMOL AND PHENACETIN

Samples	*p*-Chloroacetanilide %	
	Thin-layer procedure	Spectrophotometric procedure
Phenacetin 1	< 0·01	< 0·01
,, 2	0·15	0·15
,, 3	0·05	0·05
,, 4	< 0·01	< 0·01
,, 5	0·02	0·02
,, 6	0·10	0·12
,, 7	< 0·01	< 0·01
,, 8	0·08	0·10
Paracetamol 6 samples	each < 0·01	—

A number of other possible impurities in phenacetin and paracetamol are separated and detected by procedure I. An estimate of the approximate amounts of each present may be obtained by methods analogous to those described for *p*-chloroacetanilide. One such impurity found in several batches of paracetamol had the same running distances as *O*-acetylparacetamol and comparison with suitable standards gave the approximate contents of this impurity, samples of paracetamol Nos 1–6 giving < 0·02, 0·03, 0·02, 0·04, < 0·02, 0·09 % respectively.

Although the thin-layer procedure we have described is capable of giving an approximate estimate of the amount of *p*-chloroacetanilide in phenacetin and paracetamol, it is better suited for use as a limit test.

TABLE 3. TABLETS CONTAINING PARACETAMOL AND PHENACETIN

Samples	*p*-Chloroacetanilide as percentage of the phenacetin
Tablets phenacetin B.P.C. 1	0·04
" " " 2	0·05
" " " 3	0·01
" " " 4	0·03
" " " 5	< 0·01
" " " 6*	0·05
Tablets paracetamol B.P. 8 samples	each < 0·01
Compound tablets acetylsalicylic acid B.P.C. 1	0·02
" " " " " 2	0·7
" " " " " 3	0·06
" " " " " 4	< 0·01
" " " " " 5*	0·05
Compound tablets codeine B.P. 1	0·03
" " " " 2	0·01
" " " " 3	0·03
Soluble compound tablets codeine B.P. 1	0·03
" " " " " 2	0·05
" " " " " 3	< 0·01

* Laboratory-prepared tablets using phenacetin sample 3 (Table 2).

Compared with other procedures it is rapid, especially when more than one sample is to be examined, and it may readily be adapted to the determination of *p*-chloroacetanilide in tablets containing phenacetin or paracetamol. It has the advantage over non-chromatographic procedures that it will also indicate the presence of other impurities and thus give additional information about the purity of the phenacetin or paracetamol.

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