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Short Communication

Determination of impurities in phenacetin by high-performance liquid chromatography

M. G. QUAGLIA[1]*, P. MAZZEO[2]† and A. LOPEZ[3]

[1] *Istituto di Chimica Farmaceutica e Tossicologica dell'Università, Piazzale Aldo Moro 5, 00185 Roma, Italy*
[2] *Dipartimento Farmaco-Chimico dell'Università, Via Amendola 173, 70126 Bari, Italy*
[3] *Istituto di Medicina Legale e delle Assicurazioni dell'Università, Piazzale Aldo Moro 5, 00185 Roma, Italy*

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Introduction

Phenacetin, a common constituent of analgesic drugs, may contain some impurities. These are derived from the synthetic pathway, and increase the toxicity of analgesic preparations. The purity of phenacetin, without identification and determination of individual impurities, has been studied by thermal methods [1–9], by gas chromatography [10–11] and by spectrofluorimetry [12]. The principal impurities of phenacetin, acetanilide, *p*-chloroacetanilide and *p*-phenetidine, have been identified with chromatographic methods [13, 14] and determined by thin-layer chromatography [15, 16]. A recent report described a procedure for the determination of *p*-chloroacetanilide in phenacetin by high-performance thin-layer chromatography [17]. The present paper describes a method for the determination of acetanilide and *p*-chloroacetanilide, and for the identification of *p*-phenetidine in phenacetin by high-performance liquid chromatography, characterized by high sensitivity and accurate and precise results.

Experimental

Reagents and chemicals

Hexane and ethyl acetate were HPLC grade (Carlo Erba, Milan, Italy). All other reagents were of analytical grade and used without further purification.

* To whom correspondence should be addressed.
† Present address: Dipartimento di Chimica, Ingegneria Chimica, Materiali dell'Università, Via Assergi 4, 67100 L'Aquila, Italy.

Apparatus and conditions

Chromatography was carried out using a Waters Liquid Chromatograph Model 6000A (Waters Associates, Milford, Mass., USA). A Jasco UVIDEC-100-III detector was used at 247 nm. Samples were applied to the chromatograph by manual injection using a U6K loop valve injector (Waters Assoc.).

The mobile phase consisted of hexane–ethyl acetate (50:50 v/v). The flow rate was kept constant at 0.9 ml/min. Separation was performed isocratically using a column Poli-Column PCS-Si-100-0515 (15 × 0.45 cm) of LiChrosorb Si 100TM (5 μm) (E. Merck, Darmstadt, FRG).

Calibration curves

The calibration curves were constructed by injection of different volumes of a solution in hexane–ethyl acetate (50:50 v/v) containing phenacetin (1 mg/ml), acetanilide (0.01 mg/ml), *p*-chloroacetanilide (0.01 mg/ml), *p*-phenetidine (0.01 mg/ml) and benzophenone (0.01 mg/ml) as internal standard, and plotting the ratios of the acetanilide and *p*-chloroacetanilide peak heights to that of the internal standard against the quantities injected. The amounts examined were between 5 and 150 ng.

Analysis of phenacetin samples

A finely powdered phenacetin sample (100 mg) was dissolved (in a volumetric flask) in 10 ml of hexane–ethyl acetate (50:50 v/v), containing benzophenone (0.01 mg/ml) as internal standard. After filtration, 10 μl of this solution was injected into the chromatograph; the amounts of acetanilide and *p*-chloroacetanilide present were calculated using the calibration curves.

Results and Discussion

Figure 1 shows the chromatogram of a solution of phenacetin in hexane–ethyl acetate (50:50 v/v) containing as impurities acetanilide, *p*-chloroacetanilide and *p*-phenetidine, and (as internal standard) benzophenone. It is clearly not possible to achieve a quantitative determination of *p*-phenetidine, because of the partial superimposition of the phenacetin peak, but it can be identified in the pharmaceutical preparation.

The calibration curves for acetanilide and *p*-chloroacetanilide were linear over the range assayed, with correlation coefficients of 0.998 in each case. The minimum amount of acetanilide and *p*-chloroacetanilide detectable was 5 ng.

The method was checked using samples prepared by adding known amounts of acetanilide and *p*-chloroacetanilide to phenacetin freed of impurities by several

Table 1
Control analyses of pure phenacetin with added acetanilide and *p*-chloroacetanilide

	Acetanilide (ppm)		*p*-Chloroacetanilide (ppm)	
Sample	Added	Found	Added	Found
1	500	450	500	500
2	300	250	300	250
3	500	450	300	300
4	300	250	500	450

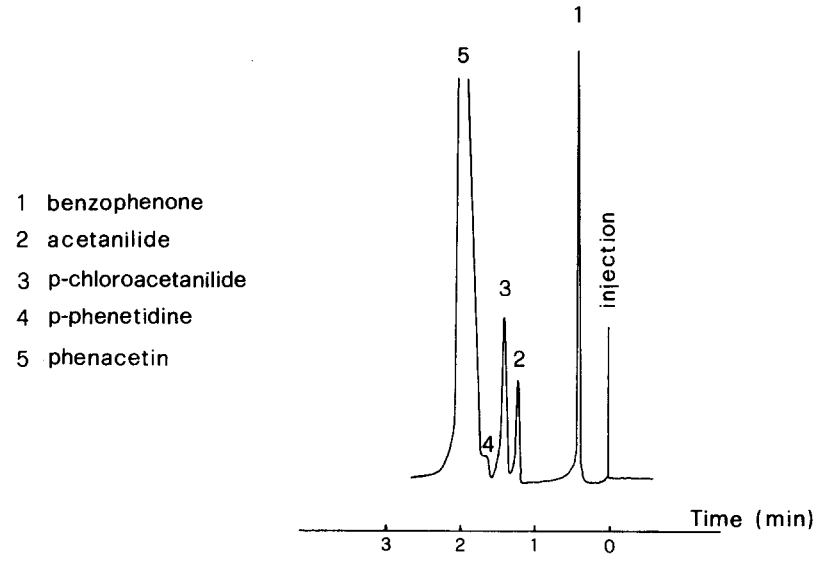

Figure 1
Chromatogram of phenacetin (1 mg/ml) in hexane–ethyl acetate (50:50 v/v) containing acetanilide (0.01 mg/ml), *p*-chloroacetanilide (0.01 mg/ml), *p*-phenetidine (0.01 mg/ml) and benzophenone (0.01 mg/ml) as internal standard. Injection 10 µl.

Table 2
Analysis of commercial samples of phenacetin

Sample	Acetanilide found (ppm)	*p*-Chloroacetanilide found (ppm)
1	—	200
2	100	300
3	50	250

recrystallizations from ethanol–water. The results are shown in Table 1. These control analyses were characterized by relative standard deviations of ca. 0.5%. Table 2 reports the results obtained in the analysis of commercial samples of phenacetin.

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