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## Note

# Rapid gas chromatographic method for emergency determination of paracetamol in human serum

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The increasing use of paracetamol as an analgesic has resulted in an increase in the number of acute cases of poisoning admitted to hospitals. Clinicians have therefore expressed the desire to have this drug analysed quantitatively by the clinical chemical laboratory on demand, 24 h a day. This desire is well motivated as overdoses of paracetamol are strongly hepatotoxic. The measured serum concentration can determine whether treatment with an antidote, in itself a somewhat critical treatment, should be initiated [1].

An analytical method that is to be used for acute determination of paracetamol must be both rapid and specific, but the sensitivity required in pharmacokinetic studies is not required in cases of poisoning.

Many methods have been described for quantitative determination of paracetamol in serum. Wiener [2] has recently given a review of methods for paracetamol estimation.

Spectrophotometric methods, which up to now have been widely used for the quantitative determination of paracetamol in serum, have not all been specific [3]. Without prior extraction the methods in which paracetamol is measured colorimetrically, following acid hydrolysis to *p*-aminophenol, will also measure inactive glucuronide and sulphate metabolites of paracetamol. The measured concentration will then be falsely elevated, perhaps leading to a misguided decision to start treatment with an antidote.

Gas chromatography (GC) is a more specific analytical method for paracetamol than spectrophotometry. Several GC methods have been described in the literature [2,4—9]. The differences between these methods are in the more or less time-consuming extraction procedures and in the use of different internal standards and different derivatising reagents. In some methods the speed

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of analysis is compromised by the occurrence of unknown peaks with long retention times [6,9].

None of the methods described combine speed of sample preparation with a rapid chromatographic analysis and none of the methods use the structural analogue 2-acetaminophenol as an internal standard. The present method for GC determination of paracetamol is characterised by a simple, rapid extraction procedure, the use of a structural analogue as internal standard and a simple, rapid procedure for derivative formation. Chromatograph settings are chosen so that samples can be injected every 3 min. There are no interfering peaks. The speed and specificity of this method make it suitable for the acute determination of serum paracetamol concentrations.

## EXPERIMENTAL

### *Apparatus*

A Pye Unicam GCD gas chromatograph equipped with flame ionisation detector was used. The 90 cm × 2 mm I.D. glass column was packed with 2.8% OV-210 and 3.2% OV-1 on Chromosorb W HP (80—100 mesh). The instrument settings were as follows: temperatures: column, 120°C; injection port, 200°C; detector, 250°C; flow-rates: nitrogen carrier, 40 ml/min; hydrogen, 40 ml/min; air, 400 ml/min; range 10 × 32 and recorder speed 10 mm/min.

### *Materials*

The following reagents were used: ethyl acetate and toluene (analytical grade; Merck, Darmstadt, G.F.R.) trifluoroacetic anhydride (Pierce, Rockford, IL, U.S.A.), paracetamol (Sterling Winthrop, New York, NY, U.S.A.), 2-acetaminophenol (pract.; Fluka, Buchs, Switzerland).

A working solution of internal standard contained 15 mg/l 2-acetaminophenol in ethyl acetate and a stock solution of 60 mmol/l paracetamol in ethanol which was stable for at least 1 year at +4°C.

Paracetamol standards containing 600, 1200 and 1800 μmol/l were prepared by adding drug-free human serum to 50-, 100- and 150-μl aliquots, respectively, of paracetamol stock solution (60 mmol/l) and making up to a total volume of 5.00 ml. The standards were stable for at least 18 months at —20°C.

### *Sample preparation*

Duplicate samples (500 μl) of standards or patient sera were pipetted into conical centrifuge tubes (100 × 16—17 mm) and 2.5 ml ethyl acetate containing the internal standard, 2-acetaminophenol (15 mg/l), added. The samples were rotated for 5 min on a blood mixer (25 r.p.m.) and centrifuged for 5 min at 600 *g*. An amount of 1.00 ml of the ethyl acetate phase was transferred to a conical tube and evaporated in a water bath at a maximum of 50°C in a stream of nitrogen. Twenty-five μl toluene and 25 μl trifluoroacetic anhydride were added to the dry residue. After vigorous mixing on a vortex mixer for 5 sec, approximately 1 μl was injected into the gas chromatograph.

## RESULTS AND DISCUSSION

The retention time for the internal standard, 2-acetaminophenol was 1.0 min under the conditions described. The retention time for paracetamol was 1.9 min (Fig. 1B). Thus a new extract could be injected every third minute.

Peak heights for standards and samples were measured. Standard concentrations (600, 1200 and 1800 μmol/l) were plotted against the ratio of paracetamol peak height to internal standard peak height. Paracetamol concentrations in patient samples were estimated from the standard curve obtained. The standard curve for paracetamol was linear up to 4000 μmol/l ($y = 0.00084x - 0.00116$; $r^2 = 0.9987$) using standards prepared from the stock solution as described in the Materials section.

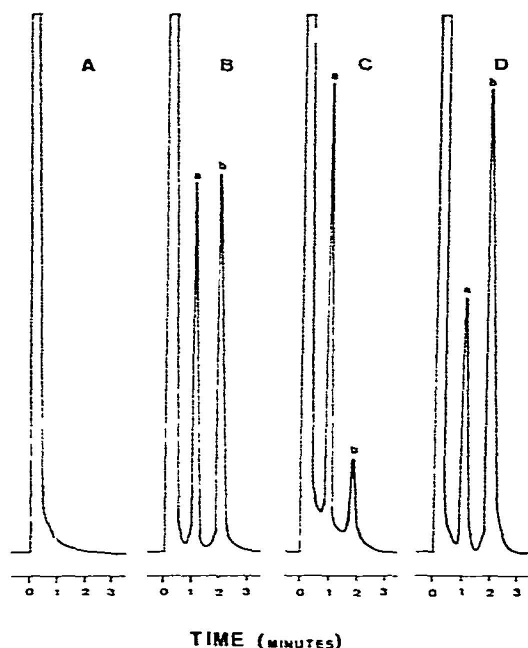


Fig. 1. GC determination of paracetamol in serum. Chromatograms of: (A) blank serum extract containing no internal standard; (B) standard extract containing paracetamol at a level of 1200 μmol/l; (C) serum extract from a patient receiving therapeutic doses of paracetamol corresponding to a serum concentration of 220 μmol/l; (D) serum extract from a poisoned patient with a serum concentration of paracetamol of 2160 μmol/l. Peaks: (a) 2-acetaminophenol (internal standard); (b) paracetamol.

The lower detection limit for the method was about 50 μmol/l. Fig. 1C shows a chromatogram of a serum extract from a patient receiving therapeutical doses of paracetamol (1 g three times a day) corresponding to a serum concentration of 220 μmol/l. Fig. 1D shows a chromatogram of a serum extract from a poisoned patient with a serum concentration of 2160 μmol/l.

The precision of the method was acceptable. The day-to-day coefficients of variation for the three standard levels (600, 1200 and 1800 μmol/l) were 4.5%, 6.6% and 4.5%, respectively ($n = 14$ at all levels). The within-run coefficient of

variation for a single extraction of 1200 μmol/l standard was 4.3% ($n$ = 8). Thus most of the method variation probably occurs during GC.

Sample preparation was a modification of the method described by Alvän et al. [4]. The sample volume and the extraction time were reduced by a factor of four. It was not necessary to extract samples for more than 5 min as longer extraction times did not improve the recovery of paracetamol.

The ratio of toluene to trifluoroacetic anhydride was 1:1 in the present method, compared with 20:1 in the method of Alvän et al. [4]. Acetylated extracts were stable at room temperature for at least 24 h.

Fig. 1A shows the chromatogram of a blank serum extract containing no internal standard. No interfering peaks were observed. The following drugs frequently found in cases of poisoning did not give interfering peaks: barbitone, phenobarbitone, morphine, codeine, ketobemidone, dextropropoxyphene, diazepam, nitrazepam, amitriptyline and nortriptyline.

Fig. 2 shows that salicylic acid, salicylamide and phenacetin gave distinct

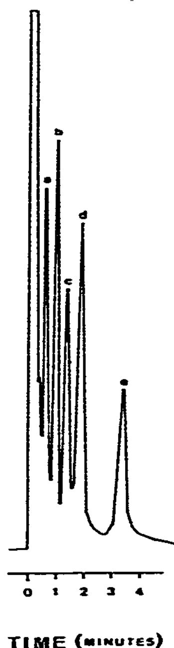


Fig. 2. Chromatogram of trifluoroacetic anhydride derivatives of (a) salicylic acid, (b) 2-acetaminophenol, (c) salicylamide, (d) paracetamol and (e) phenacetin.

peaks in the chromatogram. Furthermore these peaks are clearly separated from those of paracetamol and 2-acetaminophenol. The retention times were as follows: salicylic acid 0.6 min, salicylamide 1.4 min, phenacetin 3.4 min.

Using the structural analogue 2-acetaminophenol as internal standard it is possible to achieve a compensation for the variation in the preparation procedure. 2-Acetaminophenol has the advantage over 3-acetaminophenol used by Alvän et al. [4] in that its chromatographic peak is better separated from the paracetamol peak and that the salicylamide peak does not coincide with the internal standard peak.

A GC emergency procedure requires a gas chromatograph to be on stand-by at all times. The chromatograph used in this laboratory is also used during the day for routine samples and is thus always ready for use in an emergency situation. The column used for paracetamol analysis is also used for the determination of morphine and codeine.

Laboratories with GC equipment should use a GC method for the determination of paracetamol in serum. The present method is rapid and specific and is therefore well suited to the emergency determination of paracetamol in cases of poisoning.

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