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# DEVELOPMENT OF A RAPID SPECTROPHOTOMETER SCREENING METHOD FOR QUANTITATION OF ACETAMINOPHEN IN HUMAN SERUM

JALIL TAVAKOLI AFSHARI M.S., MT (ASCP), CLS (NCA) AND,
TZAN LIU Ph.D., MT (ASCP)

*From the Department of Laboratory Medicine, San Francisco State University, California, U.S.A.*

## ABSTRACT

We describe a simple, economical procedure for the emergency determination of serum acetaminophen levels. Prior to color development, free unconjugated acetaminophen was separated from other endogenous compounds containing phenol groups by extracting the acetaminophen into ethyl acetate and hydrolyzed to p-aminophenol by treatment with hot acid. A blue color, which can be measured at 635 nm is formed by the addition of 2,5-dimethylphenol (p-xylenol) and sodium periodate, based on the reaction of a primary aromatic amine and a phenolic hydroxyl reagent (Figure 1).

This method is linear from 25-600 mg/L. The intra-run precision had day-to-day coefficient variation of between 4.8 and 7.0. The method was correlated with established nitration method of Glynn and Kendal (1975) and the colorimetric method of Liu and Oka (1980) with the correlation coefficient of 0.97 and 0.98 respectively.

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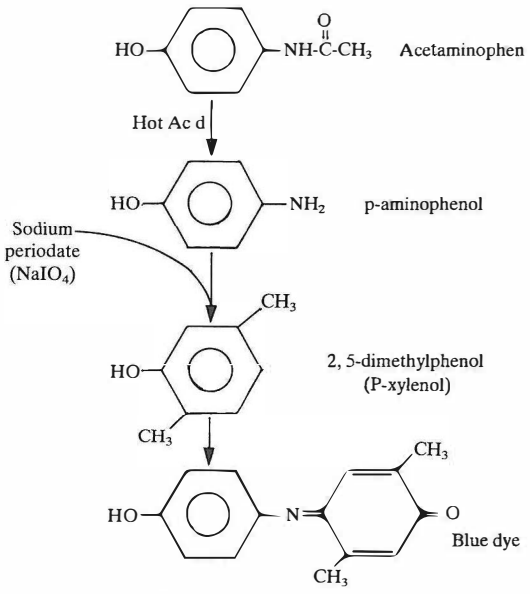


**Figure 1. Principles of the proposed method.**

## INTRODUCTION

Acetaminophen is a widely used antipyretic and analgesic agent which has been promoted as a safe alternative to aspirin. During the last few years the misuse of acetaminophen (N-acetyl-P-aminophenol) has been associated with severe and sometimes fatal hepatotoxicity,[7] and nephrotoxicity.[9]

The diagnosis and treatment of acetaminophen overdose are based largely on information concerning the acetaminophen concentration in serum and the time of drug ingestion. Overdose patients must be given an antidote within 10 hr of drug ingestion. Thus, it is imperative that the clinical laboratory provides accurate results within a short period of time. Several highly sensitive and specific HPLC and GLC methods have been reported, but they require the availability of expensive equipment and specially trained analysts.

We describe here a simple screening method for the quantitation of serum acetaminophen in cases of sus-

pected acetaminophen overdose. The proposed method requires a minimum number of reagents and can be completed within 15 minutes. The only instrument required is a spectrophotometer.

## MATERIALS AND METHODS

### Reagents

0.4M potassium hydroxide is prepared by dissolving 2.4g of potassium hydroxide (Baker Chemical Co.) in 100 mL of distilled water.

The dye reagent is prepared by dissolving 82mg of 2,5-dimethylphenol (Aldrich Chemical Co.) and 32mg of sodium periodate (Aldrich Chemical Co.) in 100 mL of 0.4M potassium hydroxide. The dye reagent is stable for one week, when kept refrigerated.

Sodium chloride crystal -(Baker Chemical Co.).

Stock acetaminophen standard, 1000 mg/L. Dissolve 100 mg of acetaminophen (Sigma Chemical Co.) in 100mL of distilled water.

Working standards.

Prepare working standards (100 and 200mg/L) by making a 10 and five-fold dilution of the stock standard with drug-free pooled serum, respectively. Store aliquots of working standards in the freezer when not in use.

### Procedure

Pipette 0.2mL of standards, controls and unknowns into 10×75mm glass tubes. Add 0.5mL of ethyl acetate and approximately 0.1g of sodium chloride crystals. Mix each tube vigorously for 30 sec using a vortex mixer.

Pipette 0.2mL of the upper organic layer into 12×100mm glass culture tubes. Add 0.1mL of 6N HCL and place the tubes into a boiling water bath for 10 minutes under a fume exhaust hood.

Remove the tubes from the boiling water bath and cool under a cold running tap. Add 3mL of dye reagent and mix.

Set the spectrophotometer to a wavelength of 635 nm and zero the absorbance against the reagent blank. Measure the absorbance within 3 hours after the addition of the dye reagent.

## RESULTS

### Absorption Spectrum

The absorption spectrum for the reaction was determined with a scanning spectrophotometer for two different acetaminophen concentrations, 50 and 100mg/L (Figure 2). Both absorption spectra exhibit a maximum absorbance at 635 nm. The absorbance of the reaction is stable from 1 to 180 minutes.

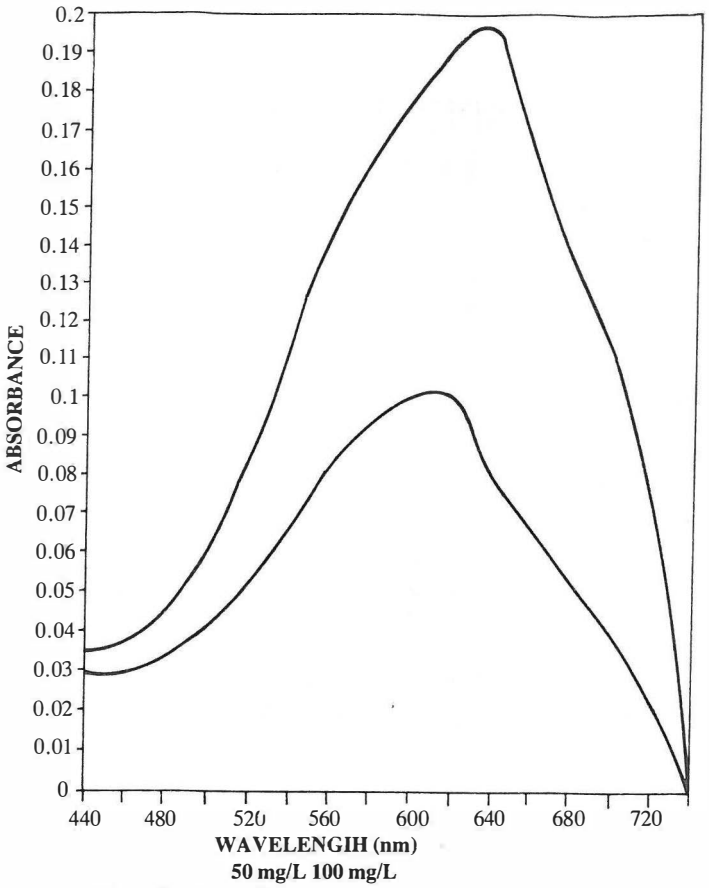


**Figure 2. Absorption spectrum for proposed method.**

### Linearity

This method follows Beer's Law from 50 to 600mg/L (Figure 3). The linear range covers both the non-toxic and toxic concentrations of acetaminophen.

### Precision

The reproducibility of the method, either day-to-day or within runs was very good (Table I). The relative variability about the mean of both controls was within acceptable limits.

### Analytical recovery

A baseline serum pool spiked with acetaminophen to a concentration of approximately 50mg/L was prepared. Various amounts of a 500 and 1000mg/L stock standards were added to aliquots of the baseline pool and subsequently assayed using the proposed method. Percent recovery was calculated by dividing the amount recovered by the original amount of acetaminophen added and multiplying by 100. The mean percentage recovery for 12 samples was 98% with a standard deviation of 3.4% (Table II).

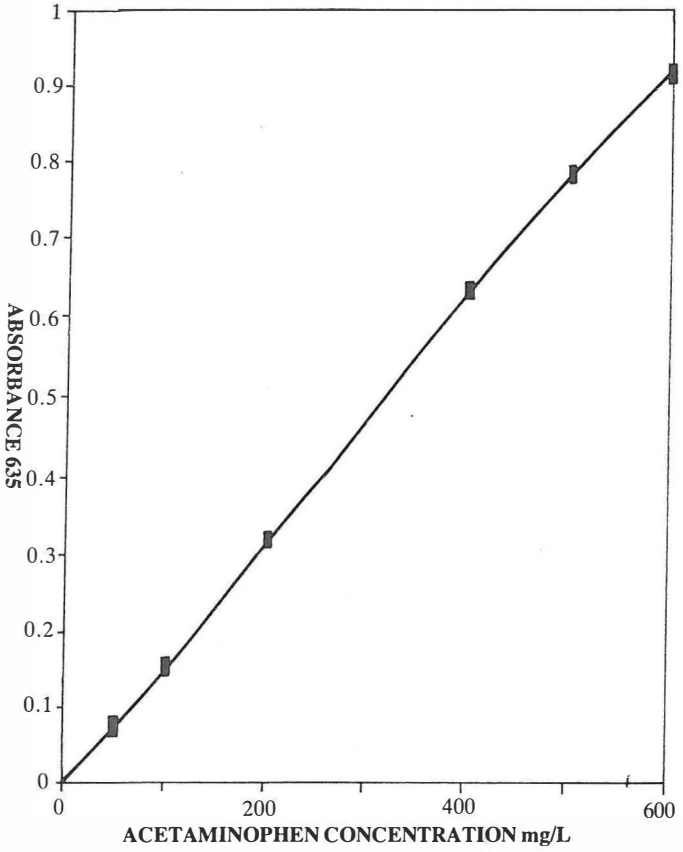


Figure 3. Linearity study for proposed method.

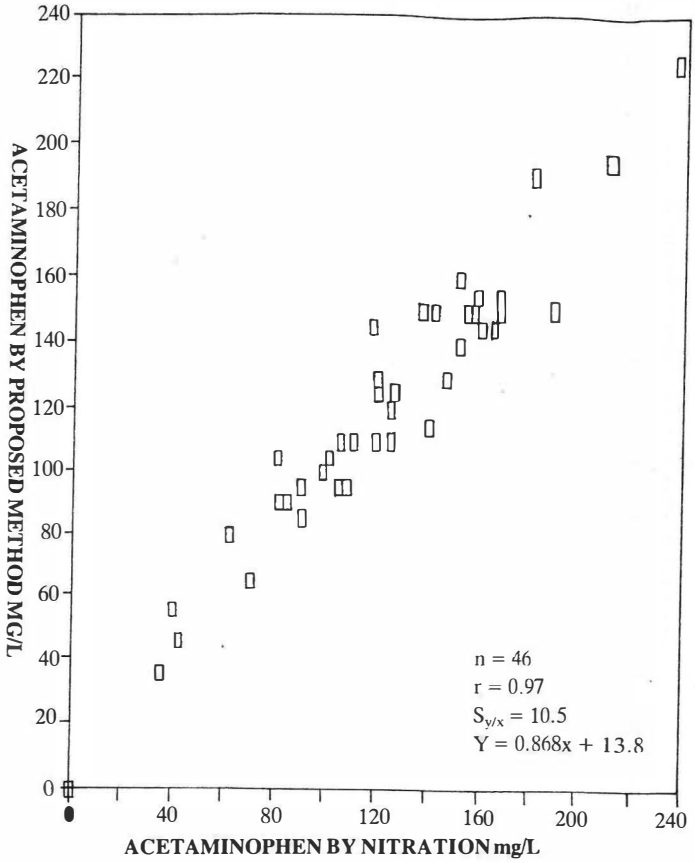


Figure 4. Correlation study nitration method vs proposed method.

Table I. Precision studies for day-to-day and within-run variability.

| Day-To-Day | Control I | Control II |
|---|---|---|
| Mean (mg/L) | 101 | 204 |
| S.D. (mg/L) | 7.0 | 9.7 |
| C.V. (%) | 7.0 | 4.8 |
| n | 40 | 40 |
| **Within-Run** | **Control I** | **Control II** |
| Mean (mg/L) | 97 | 193 |
| S.D. (mg/L) | 4.5 | 3.2 |
| C.V. (%) | 5 | 5 |

Table II. Recovery Studies

| Concentration Measured | Concentration Added | Concentration Recovered | Recovery % |
|---|---|---|---|
| 25* | 0 | --- | --- |
| 73 | 50 | 48 | 96 |
| 76 | 50 | 51 | 102 |
| 123 | 100 | 98 | 98 |
| 120 | 100 | 95 | 95 |
| 160 | 143 | 135 | 94 |
| 164 | 143 | 139 | 97 |
| 195 | 175 | 170 | 97 |
| 208 | 175 | 183 | 105 |
| 221 | 200 | 196 | 98 |
| 215 | 200 | 190 | 95 |
| 264 | 250 | 239 | 96 |
| 281 | 250 | 256 | 102 |

* mg/L (Baseline)
Note: average recovery = 98%: S.D. = 3.4%

## Correlation studies

We compared results of the proposed method with the nitration method of Glynn and Kendal[1] (Figure 4), for which the regression line was y=0.87X +13.8 and the correlation coefficient was 0.97, and the DAFR dye method of Liu and Oka[8] (Figure 5), for which the correlation coefficient was O.98 and the regression line was y= O.96X − 1.4. Comparison of data obtained by our proposed method against those by the Emit method[13] for 35 samples yields a correlation coefficient of O.87 with y = O.77+27.4 (Figure 6).

## Drug interference studies

Twenty five common drugs were added to

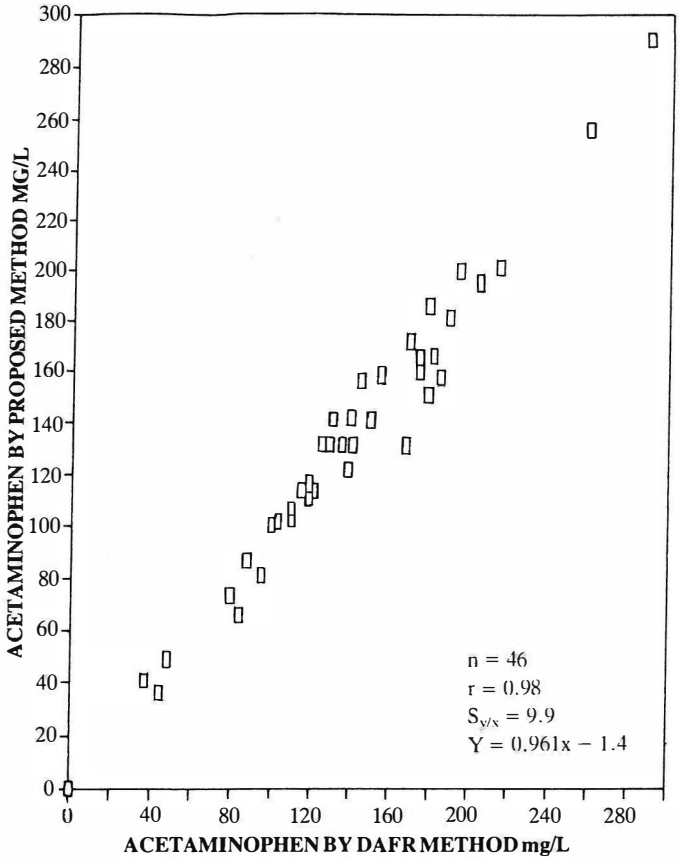


**Figure 5. Correlation study, dafr vs proposed method.**

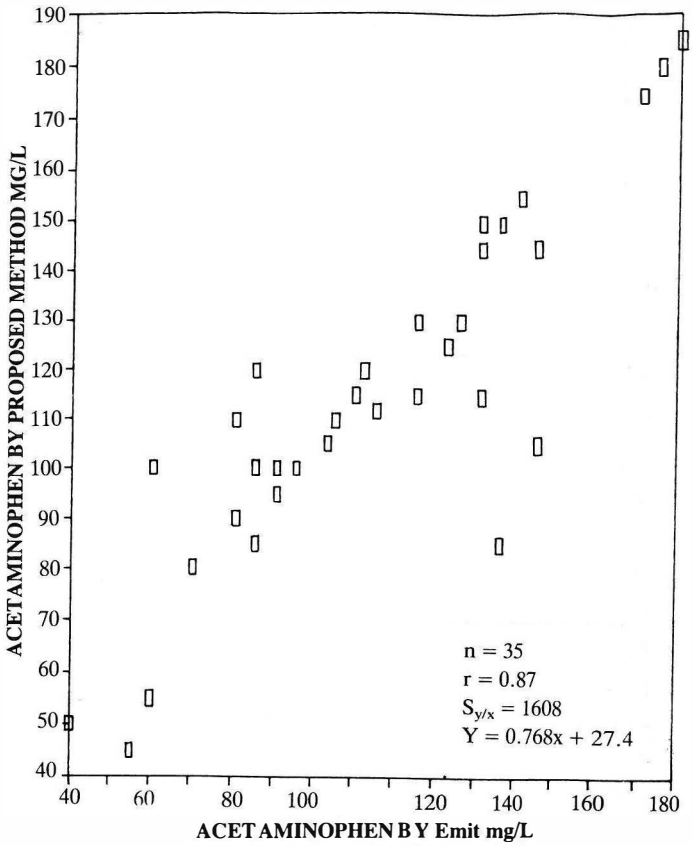


**Figure 6. Correlation study, emit vs proposed method.**

acetaminophen-free serum and tested with the proposed method. Included were common drugs of abuse and drugs often taken concomitantly with acetaminophen or available in propietary mixture with acetaminophen. The dye reagent reacts specifically with drugs containing or yielding, following hydrolysis, a primary aromatic amine. Drugs such as phenacetin and procainamide may interfere after ingestion. Phenacetin, available in proprietary analgesic mixtures, has a half life of one hour and is extensively metabolized to acetaminophen by dealkylation (Prescott, 1980).

## DISCUSSION

Clinical diagnosis and management of acetaminophen overdose would be greatly enhanced if a rapid, reliable screening method for this drug were available. Acetylcysteine, the current treatment of choice, appears to be less toxic and better tolerated than previous antidotes. Results of clinical studies suggest that if serum samples and assay results are available within 16 hours post-ingestion, laboratory confirmation of acetaminophen toxicity should be made before treatment. However, clinicians are advised to begin treatment in cases of suspected overdose when blood levels are not available within the specific time limit.

We believe that the present method meets the current need for a fast, simple, reliable assay procedure for acetaminophen blood level determination. The proposed dye method requires a minimum number of reagents and all measurements are made on a standard spectrophotometer. This procedure is linear up to an acetaminophen concentration of 600mg/L; both the therapeutic and toxic concentrations are included.

The proposed method has excellent recovery: it is quick and economical and requires only 30 seconds extraction. Inclusion of the extraction step makes this method specific for free, clinically significant acetaminophen and eliminates measurment of absorbance due to the conjugated forms of acetaminophen. The total analysis time for one to 12 samples was less than 30 minutes.

The ease of reproducibility, convenience, simplicity, and small sample volumes along with the good specificity and sensitivity make this a suitable method for routine hospital clinical laboratories. However, it must be kept in mind that the purpose of a statistical evalution is to show the magnitude of errors present. Judging acceptability is a subjective process, which is often difficult. Therefore, this decision is left to the

clinical and technical staff of each individual laboratory.

## REFERENCES


1. Glynn, J.P., and Kendal, S.E.: Paracetamol measurment. Lancet I,1147, 1975.
2. Koch-Weser, J: Acetaminophen. N.Engl. J.Med. 295:1297-1300; 1976.
3. Ahmad, A: Modified enzymatic assay for acetaminophen. Clin. Chem. 32:699-700,1986.
4. Blair, D. and Rumack, B.H: Acetaminophen in serum and plasma estimated by high pressure liquid chromatography: A microscale method. Clin. Chem. 23:743-745,1977.
5. Campbell, R.S. and Hammhond, P.M: The measurment of serum paracetamol (acetaminophen) using a discrete analyzer. J. Autom. Chem. 5:146-149, 1983.
6. Devalia, J.L. and McLean, A.E.M: Covalent binding and the mechanism of paracetamol (acetaminphen) toxictity. Biochem. Pharmacol. 32:2602-2603, 1983.
7. Hall. A.H. and Rumack, B.H: Managment of acute acetaminophen overdose. Am.Fam. Physician 33:107-114,1986.
8. Liu, T.Z. and Oka, K.H: Spectrophotometric screening method for acetaminophen in serum and plasma. Clin. Chem. 26:69-71, 1980.
9. Meredith, T.J. and Prescott, L.F: Why do patients still die from paracetamol (acetaminophen) poisoning? Br. Med.J. 293: 345-346,1986.
10. Rumack, B.H: Acetaminophen: Acute overdose toxicity in children. Drug Intell. Clin. Pharm. 138:911-912, 1985.
11. Stewart, M.J. and Willis, R.G: Simplified gas chromatographic assay for paracetamol. Ann. Clin. Biochem. 12:4-8,1975.
12. Walberg, C.B: Determination of acetaminophen in serum. J. Anal. Toxicol. I: 79-80,1977.
13. Syva Co, California, Emit Homogenous assay, 1983.