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Spectrophotometric determination of paracetamol in pure form and in tablets

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Abstract: A spectrophotometric method is proposed for the determination of paracetamol in pure form and in tablets. The method depends on reaction of the drug with ammonium molybdate in strongly acidic medium to produce molybdenum blue. Effects of variables such as temperature, heating time, acidity and reagent concentration have been evaluated to permit selection of the most advantageous technique. Beer's law was followed for concentrations of up to 6 μg ml^{-1} of paracetamol and the detection limit ($p = 0.05$) was 0.10 μg ml^{-1}. The molar absorptivity at 670 nm was 2.6×10^4 l mol^{-1} cm^{-1} and the relevant Sandell's sensitivity of the reaction was 0.0059 μg cm^{-2} per 0.001 absorbance unit. Statistical analysis of the results and comparison with results by the BP method of analysis are also reported.

Keywords: *Paracetamol determination; ammonium molybdate; spectrophotometry.*

Introduction

Paracetamol (4′-hydroxyacetanilide) is an extensively employed analgesic and antipyretic. The most common dosage forms for paracetamol are tablets. Paracetamol is equivalent to aspirin in terms of analgesia and antipyretic potency; since it rarely produces gastrointestinal irritation, it is used as an aspirin substitute for those individuals who suffer gastric discomfort with aspirin. Overdose of paracetamol causes hepatic necrosis; blood concentrations over 300 ppm of the drug 4 h after administration, cause serious hepatic damage; hence, accurate analytical methods for the assay of paracetamol are needed.

Many methods for its determination have been described. In the BP method [1] paracetamol is determined titrimetrically with ammonium cerium(IV) sulphate in ice, after boiling the drug under reflux for 1 h, using ferroin as indicator. Other recent methods include: reaction with potassium hexacyanoferrate(III) at 35°C and subsequent iodimetric titration of the excess of the reagent [2]; a normal-phase high-performance liquid chromatography (HPLC) system using a microparticulate silica column and a modified butyl chloride mobile phase [3]; a flow injection–spectrophotometric procedure based on oxidation of the drug with potassium hexacyanoferrate(III) and reaction of the *N*-(hydroxyphenyl)-*p*-benzcquinonimine produced with phenol (both reactions at 80 ± 1°C) [4]; spectrophotometric determination using 2-iodylbenzoate as reagent (molar absorptivity, 651 l mol^{-1} cm^{-1} at 444 nm) [5]; reaction of paracetamol with iodylbenzene (synthesized by a time-consuming procedure) followed by spectrophoto-

metric determination of the yellowish orange *N*-acetyl-1,4-benzoquinonimine produced (molar absorptivity, 1.58×10^3 l mol^{-1} cm^{-1} at 430 nm) [6]; spectrophotometric determination with cerium(IV) sulphate after heating at 80°C for 90 min (molar absorptivity 811 l mol^{-1} cm^{-1} at 410 nm) [7].

In previous papers, spectrophotometric methods for quantitation of certain classes of antibiotics [8–10, 12] have been described, based on their oxidation with ammonium molybdate. As part of a continuing investigation on analytical procedures for products of clinical interest [8–13], the reduction of molybdate to molybdenum blue by paracetamol was also investigated.

The purpose of this study was to develop an accurate and sensitive spectrophotometric procedure for the determination of this drug, which compares favourably with other recent spectrophotometric methods, in terms of simplicity and/or sensitivity.

The possibility of using the present method for the determination of paracetamol in tablets was also explored.

Statistical analyses of the experimental data are presented and the present method is compared with the BP methods of analysis for both the pure drug [1] and the tablets [14].

Experimental

Reagents

Paracetamol solution. A freshly prepared solution containing 0.25 mg ml^{-1} of paracetamol in water. The paracetamol was provided by Lepetit S.p.A., Italy.

Drug formulations. Tablets of Acetamol (Istituto Gentili S.p.A., Italy), Panadol (Winthrop S.p.A., Italy) and Tachipirina (Angelini S.p.A., Italy) all 500 mg paracetamol in each tablet, were obtained locally.

Ammonium molybdate solution. A solution containing 25% m/v of ammonium molybdate in 50% v/v sulphuric acid.

Equipment

All absorbance measurements were made in the double-beam mode with a Perkin–Elmer 555 spectrophotometer using 1-cm quartz cells.

General procedure

A volume of drug standard solution expected to contain up to 30 μg of paracetamol is mixed with 2.5 ml of ammonium molybdate solution and 1.8 ml of concentrated sulphuric acid in a 5-ml calibrated flask, cooled to room temperature and diluted to 5 ml with distilled water. The mixture is allowed to react in a water-bath at 99°C for 80 min; the reaction is then quenched by cooling for about 2 min in ice-water. The absorbance of the coloured product is measured at 670 nm against a reagent blank prepared under the same conditions.

The colour of the solutions is stable for at least 1 h at room temperature (measurements were not made after 1 h).

Procedure for tablets

With tablets it is necessary first to isolate paracetamol from the excipients.

Three weighed tablets are ground into a fine powder in a mortar. A mass of powder

containing about 500 mg of paracetamol is accurately weighed, mixed with 50 ml of water and stirred for 10 min. The insoluble residue is removed by filtration through a Whatman No. 41 filter-paper and thoroughly washed with water. The extract (with washings) is evaporated to dryness under vacuum and working solutions of paracetamol are prepared by dissolving the requisite amount of drug in distilled water.

Alternatively, the filtrate (with washings), collected in a 500-ml calibrated flask, is diluted to volume with water and suitable aliquots of solution taken for analysis. Since this procedure eliminates the need for evaporation to dryness, it is less time consuming; however, the two procedures give similar results. The assay is completed as described above. The paracetamol content in the formulations is computed from a regression equation derived from analysis of standard solutions of the pure drug.

Results and Discussion

Although the reaction, with molybdate, of certain drugs was previously studied [8–10, 12], the effects of different variables were verified in order to develop a simple and convenient procedure for the determination of paracetamol and to optimize the conditions.

The method was repeatedly carried out using different concentrations of paracetamol; the results were independent of the concentration of paracetamol.

Effect of temperature and heating time

Samples prepared as described above were measured at 670 nm at various temperatures as a function of time of heating. The blue compound was not formed at room temperature; the reaction rate was enhanced at elevated temperatures and maximum absorbance occurred after heating for about 80 min at 99°C. Further heating caused no appreciable increase of intensity of the colour. Measurements at 94°C (80 min heating time) showed a 15% decrease in absorbance; hence a development time of 80 min at 99°C was optimum for all experiments.

A set of experimental curves in the range 70–99°C, obtained with samples of 3.8 μg ml^{-1} of paracetamol, is shown in Fig. 1.

Effect of sulphuric acid concentration

A typical curve of absorbance against sulphuric acid concentration in the final 5-ml solution, obtained with samples of 2.8 μg ml^{-1} of paracetamol, is shown in Fig. 2. An 11 M concentration of sulphuric acid gave the most sensitive results; use of lower and higher concentrations of sulphuric acid resulted in lower absorbance values.

Effect of molybdate concentration

Figure 3 shows the effect on the absorbance of varying the volume of ammonium molybdate solution (1.0 to 2.7 ml in the 5-ml samples). The curve has been plotted with samples of 2.1 μg ml^{-1} of paracetamol. The absorbance increases up to about 2.4 ml of molybdate solution, then it remains almost constant. A volume of 2.5 ml was selected as suitable; this corresponds to a final concentration of molybdate in the samples of about 12.5% m/v.

Absorption spectra

In Fig. 4a are shown two spectra developed under the specified conditions of assay

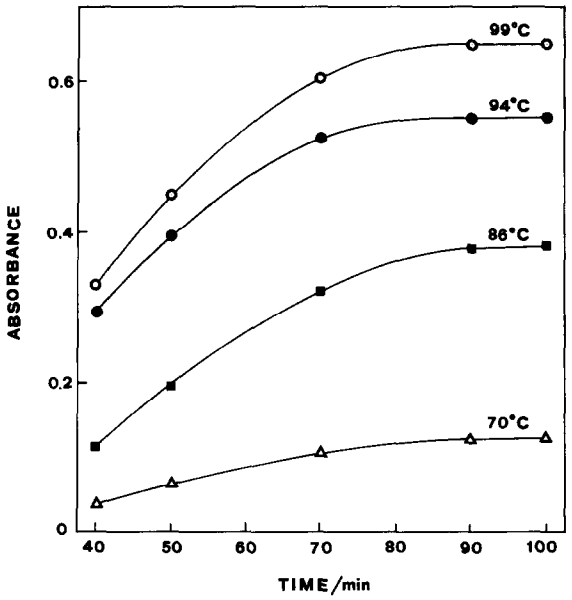

Figure 1
Effect of temperature on absorbance of the coloured reaction product. 3.8 μg ml^{-1} of paracetamol; 670 nm; reference, reagent blank.

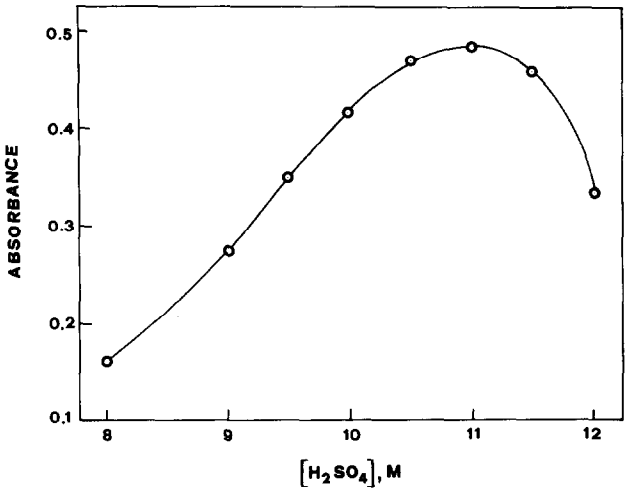

Figure 2
Effect of sulphuric acid concentration on absorbance of the coloured reaction product. 2.8 μg ml^{-1} of paracetamol; 670 nm; reference, reagent blank.

with samples of 3.0 μg ml^{-1} (curve 1) and 5.5 μg ml^{-1} (curve 2) of paracetamol, respectively.

The spectra, with a maximum absorbance at 670 nm, were attributed to molybdenum blue formed by reduction of molybdenum(VI) by the drug [8–10, 12].

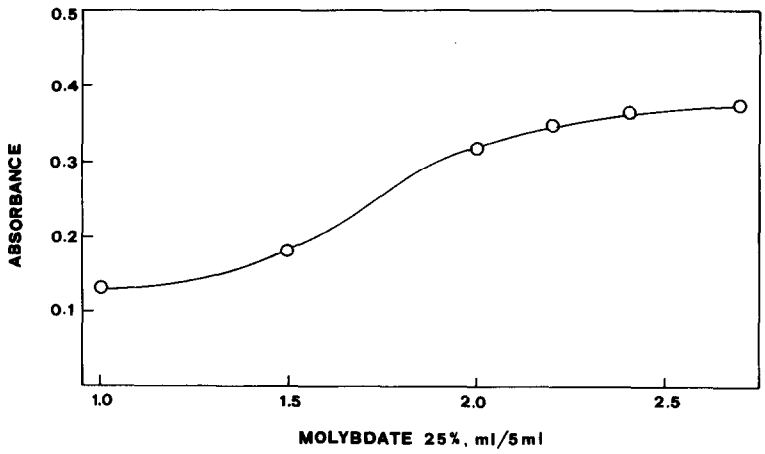

Figure 3
Effect of ammonium molybdate concentration on absorbance of the coloured reaction product. 2.1 μg ml^{-1} of paracetamol; 670 nm; reference, reagent blank.

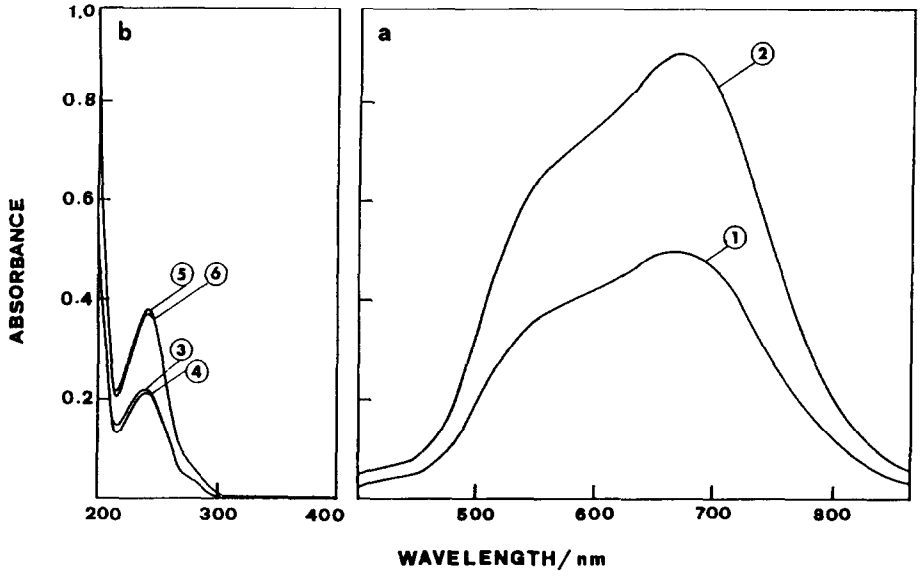

Figure 4
(a) Absorption spectra of the coloured product of the reaction of paracetamol with molybdate under optimum conditions. Curve 1, 3.0 μg ml^{-1} of paracetamol; curve 2, 5.5 μg ml^{-1} of paracetamol; reference, reagent blank. (b) Absorption spectra of paracetamol alone with (curves 4 and 6) and without (curves 3 and 5) treatment at 99°C for 80 min. Curves 3/4, 3.0 μg ml^{-1} of paracetamol; curves 5 and 6, 5.5 μg of paracetamol; reference, water.

In Fig. 4b the corresponding direct UV spectra of paracetamol alone are shown (curves 3 and 4, 3.0 μg ml^{-1}; curves 5 and 6, 5.5 μg ml^{-1}). Curves 3 and 5 were obtained at room temperature (without heating); curves 4 and 6 were obtained after heating at 99°C for 80 min as in the general procedure. Comparison of Figs 4a and 4b shows a large gain in sensitivity by use of the present method.

Statistical treatment of experimental data

Samples of paracetamol at a range of concentrations up to 6 μg ml^{-1} were assayed by the proposed method. Beer's law was obeyed over the range.

The regression equation is $A = 0.0033 + 0.1696\ c$ (A, absorbance at 670 nm, 1-cm cell; c, concentration, μg ml^{-1}). The equation was calculated from calibration data, with 12 samples containing 0.5–6.0 μg ml^{-1} of paracetamol. The correlation coefficient is 0.9996. The molar absorptivity is 2.6×10^4 l mol^{-1} cm^{-1} and the relevant Sandell's sensitivity of the reaction was calculated to be 0.0059 μg cm^{-2} per 0.001 absorbance unit.

The variance and detection limit (p = 0.05) for $n - 2 = 10$ d.f., were calculated to be 7.2×10^{-5} and 0.10 μg ml^{-1}, respectively, which confirm the sensitivity of the method and the negligible scatter of the points with respect to the line of regression.

It is important to verify if the intercept, a, of the line of regression is significantly different from zero.

A simplified method of estimating the difference $a - 0$ is based on the determination of the quantity $t = a/s_c$ [15] and its comparison with the corresponding tabular data for the t-distribution. The value calculated (0.63) does not exceed the 95% criterion ($t_p = 2.23$) which means that the calculated intercept is not significantly different from zero; thus the present method is free from constant errors independent of the concentration of paracetamol.

However, a more rigorous approach requires the construction from calibration data of a joint confidence region for slope, b, and intercept, a (because of the strong correlation existing between these parameters), which is an ellipse having as centre the least-squares estimates of a and b.

This is drawn in Fig. 5, by following the method of Mandel and Linning [15, 16].

From an inspection of Fig. 5 it is evident that points for which the intercept is zero, fall well inside the ellipse, confirming the above conclusions.

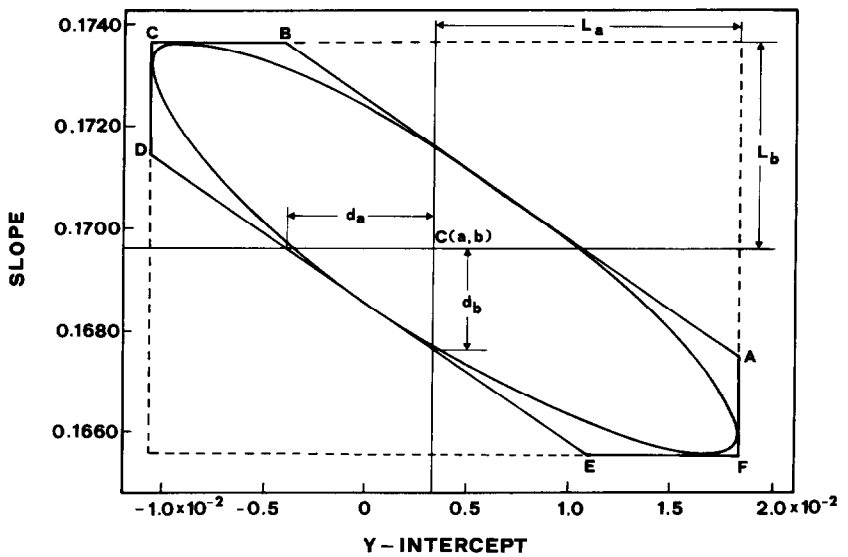

Figure 5
Joint confidence region (p = 0.05) for slope and intercept of line of regression of paracetamol ($n = 12$).

Figure 6 shows the histograms of the absolute error, s_c [15], in the determination of a given concentration, calculated by means of statistical analysis of the regression equation of pure paracetamol.

The error is a minimum for a concentration of 3 μg ml^{-1} of paracetamol.

Accuracy and precision

Five replicate determinations of each sample solution were carried out to test the accuracy and precision of the method for the determination of paracetamol in its pure form and in a number of tablets (after extraction of paracetamol, as previously described). The results appeared to be highly satisfactory. These are reported in Table 1 together with the results obtained by the BP methods for paracetamol in pure form, i.e. titration with ammonium cerium(IV) sulphate [1], and for tablets, i.e. UV measurement of a 1-cm layer of the solution, taking 715 as the value of E (1%, 1 cm) at 257 nm [14].

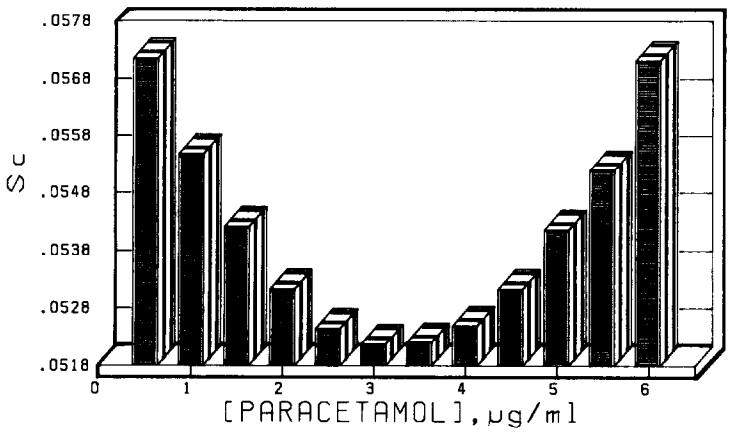

Figure 6
Histograms of the error in the determination of the concentration (μg ml^{-1}) of paracetamol (n = 12).

Table 1
Results of the determination of paracetamol in pure form and in tablets, compared with those by the BP methods

Drug or proprietary tablets	Composition	Recovery%* ± Standard deviation Present method	BP methods [1, 14]	t (calculated)†
Paracetamol, laboratory made (Lepetit)	100.50% Paracetamol (absolute)	100.48 ± 0.13	100.49 ± 0.11	0.75
Panadol tablets (Winthrop)	500 mg Paracetamol	100.97 ± 0.13	101.19 ± 0.13	2.59
Tachipirina tablets (Angelini)	500 mg Paracetamol	100.29 ± 0.15	100.66 ± 0.62	1.37
Acetamol tablets (Istituto Gentili)	500 mg Paracetamol	100.11 ± 0.10	100.47 ± 0.75	1.02

*Mean of five determinations, assay as percentage of label claim.
†Theoretical value of t (p = 0.05) = 2.78.

From statistical analysis of the findings of the present method and of pharmacopoeial methods [1, 14], the calculated *t*-value at the 95% confidence level did not exceed the theoretical t_p value (see Table 1); therefore the null hypothesis is verified, indicating no significant differences from the results of the BP methods. However, the proposed method is regarded as being superior, particularly in relation to the BP method for paracetamol in pure form, which is titrimetric, hence poorly sensitive. Moreover, also taking into account the results of the *t*-test, the proposed method is considered to be a little superior to the BP tablet assay too; the proposed method gives, in addition to high sensitivity (which, really, is of no great importance in the assay of tablets), slightly more accurate and reproducible results (Table 1). In fact, although the BP UV spectrophotometric assay for tablets is simple (comparable with the present method), it is well known that its accuracy may be influenced by interference from diluents and binders in tablets. This is shown, for example, by the results of the BP assay method for Panadol and the corresponding value of *t* (2.59), which is not very different from the theoretical value (2.78).

Conclusions

The procedure is very simple and involves a minimum number of reagents and reaction sequences.

The reproducibility and accuracy of the results are very satisfactory; the sensitivity is sufficiently high and compares favourably with that of other recent spectrophotometric methods for paracetamol [5–7].

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