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COMPUTER-ASSISSTED SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF ACETAMINOPHEN AND PHENACETIN IN THE PRESENCE OF THEIR DEGRADATION PRODUCTS USING COMBINED ORTHOGONAL POLYNOMIALS.

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ABSTRACT

A computer-assissted spectrophotometric method for determining acetaminophen and phenacetin in presence of their degradation products is presented. The computer program (CNVSPCI) provides a very wide choices during optimization of the combined orthogonal polynomial ceofficients calculated for the analytes (acetaminophen or phenacetin in the presence of their respective degradation products-p-aminophenol and p-phenetidine). The proposed method has been proved to be stability-indicating method since graphs of log C% VS time were found linear for both drugs.

INTRODUCTION

Acetaminophen hydrolysis to p-aminophenol and acetic acid[1] using different hydrochloric acid concentrations and buffered (pH2-9) aqueous solutions has been investigated[2]. Meanwhile phenacetin (acetophenetidine) is susceptible to hydrolysis in acid medium to p-phenetidine and acetic acid[3]. Recently, the derivative spectrophotometry has been used for determining acetaminophen and phenacetin in the presence of their degradation products[4].

The combined polynomial method[5] has been lately used to the determination of certain drugs like thiamine hydrochloride[6], procaine hydrochloride[7], some penicillines[8], and methylphenobarbitone[9].

The combined polynomial (P_w) is given by the following formula[5].

$$P_{wi} = \frac{ap_{ji}\ N_k/F + b\ P_{ki}\ N_j/F}{N_j\ N_k/F} \qquad (1)$$

where a and b are integers, F is a common factor used to bring the numerator to the simplest integers, subscripts j and k stand for different polynomials, i is the set of wavelengths, N is the normalizing factor and P is the polynomial. The coefficient, p_w, of the combined polynomial, P_w, can be obtained by

$$p_w = \sum_{i=0}^{n} A_i . P_{wi}/D \qquad (2)$$

where P_w stands for $ap_j + bp_k$.

Accordingly for a pure compound, X

$$p_w = \alpha_w\ C_x$$

where α_w is the combined polynomial coefficient-a substitute of A (1%, 1 cm)-of compound X.

In the presence of irrelevant absorption which contributes to p_j and p_k, each observed combined coefficient, p_w, is the sum of two terms, thus

$$p_w = \alpha_w\ C_x + p_w\ (z) \qquad (3)$$

where $p_w\ (z) = ap_j\ (z) + b_k\ (z)$.

Arranging for the numerical values and signs of a and b (to be such that p_w (z) becomes negligibly small relative to

$\alpha_w C_x$) the concentration of compound X can then be obtained from p_w.

In the present work a computer program (CNVSPC 1, described in Fig. 1)[10] has been described to provide a very wide choices helpful in convoluting the absorption curves of both the drug (x) and its degradation products or any interfering component (z). This could be accomplished through the use of (a) different number of polynomial points, (b) different polynomial orders and(c) different wavelength intervals. The λ_m was then located at which the coefficients p_j and p_k could be maximized for x and minimized for z.

EXPERIMENTAL

Instrument:

A Perkin-Elmer 550S ultraviolet spectrophotometer.

Materials & Reagents.

p-Aminophenol solution, transfer accurate weight of 50 mg p-aminophenol into 100 ml calibrated flask. Dissolve and complete to volume with 0.1 N hydrochloric acid. Dilute 2 ml to 100 ml with 0.1 N hydrochloric acid solution.

p-Phenetidine solution, transfer accurate weight of 100 mg phenacetin into 100 ml conical flask. Add 10 ml hydrochloric acid and reflux for 30 min, over boiling water bath. Cool to room temperature and wash the condenser with 10 ml water.Transfer quantitatively to 100 ml volumetric flask and complete to volume with water. Dilute 1.0 ml to 100 ml with 0.1 N hydrochloric acid.

Preparation of calibration curves for acetaminophen and phenacetin.

Transfer and dissolve accurate weights of 100 mg acetaminophen and phenacetin into separate 100-ml volumetric flasks using about 5 ml methanol. Complete to volume with 0.1 N hydrochloric acid (Stock solution). Prepare 6 concen-

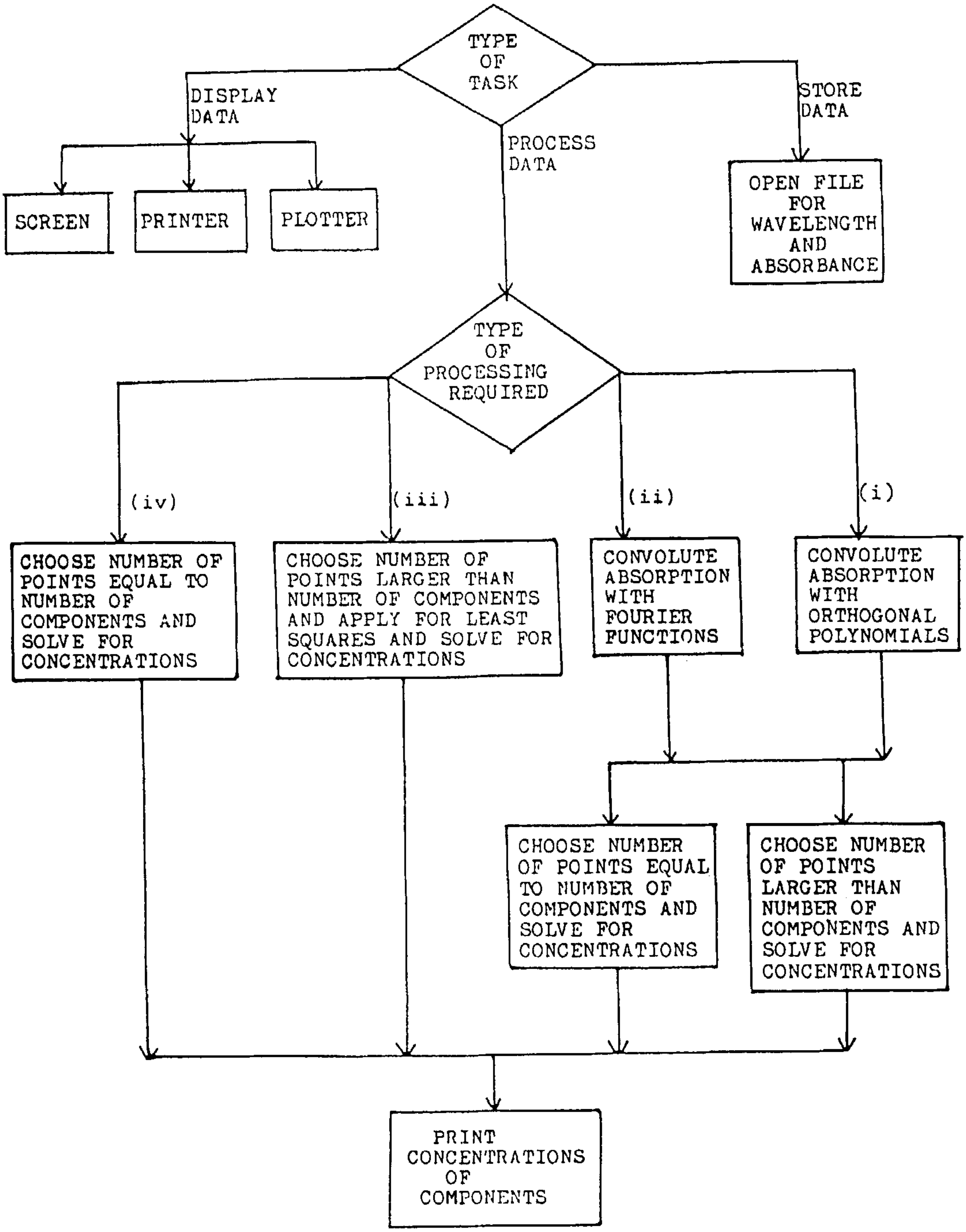

FIGURE 1

Functional blockdiagram of computer program.

trations from each stock solution in the range of 0.4-1.4 mg/100 ml (in 0.2-mg steps) by quantitative dilution of solution with 0.1 N hydrochloric acid. Measure the absorbance in 1-cm cell in the range 238 to 260 at 2-nm intervals using 0.1 N hydrochloric acid solution as blank. Calculate the coefficient p_w of the combined polynomial P_W for each set of twelve absorbances.

Stability Investigations of Acetaminophen and Phenacetin.

Transfer and dissolve accurate weights of 100 mg of acetaminophen or phenacetin into separate 50-ml volumetric flask using about 5 ml methanol. Complete to volume with 6 N hydrochloric acid. Put in a thermostated water bath of different temperatures (50°C, 70°C & 90°C) and leave to equilibrate for 5 min. Thereafter transfer 1.0 ml aliquots at different time intervals to separate 250-ml calibrated flasks. Complete to volume with 0.1 N hydrochloric acid. Measure the absorbances of each sample in the range of 238 to 260 nm at 2-nm intervals using 0.1 N hydrochloric acid as blank. Using the computer program calculate the coefficients $p_w = (p_2 + 10\ p_3)$ and determine the drug concentration at each time intervals.

Determination of acetaminophen in pharmaceutical preparations.

Preparation of sample solution.

Tablets: Transfer accurate weight of finely powdered commercial (Paracetamol/Noflu) tablets equivalent to 50 mg acetaminophen into 100-ml volumetric flask containing about 50 ml 0.1 N hydrochloric acid. Shake for 15 min and complete to volume with 0.1 N hydrochloric acid. Filter, discarding the first 2-3 ml. Using 2.0 ml filterate proceed according to the following general procedure.

Syrup: Pipette 2.0 ml of commercial (Paracetamol) syrup equivalent to about 50 mg acetaminophen into 100-ml volumetric flask and complete to volume with 0.1 N hydrochloric acid. Using 2.0 ml of the prepared solution, proceed according to the following general procedure.

Suppositories: Weigh the contents of six commercial (Grippo) suppositories, melt in a clean porcelain dish over boiling water bath and allow to cool to room temperature ($\sim 20^{\circ}C$). Transfer accurate weight of suppositories mass equivalent to 100 mg acetaminophen into a clean dry 25-ml beaker. Extract with successive volumes of hot methanol(20,10&10 ml) and transfer quantitatively to 50-ml volumetric flask. Cool and complete to volume with methanol. Dilute 25.0 ml of the methanol extract to 100.0 ml with 0.1 N hydrochloric acid. Using 2.0 ml of the prepared solution, proceed according to the following general procedure.

General procedure of assay.

Dilute 2.0 ml portion of the final prepared sample solution to 100.0 ml with 0.1 N hydrochloric acid. (i) Measure the absorbances in the range of 238 to 260 nm, at 2-nm intervals using 0.1 N hydrochloric acid blank. Calculate the coefficient $p_w = (p_2 + 10p_3)$ (ii) Calculate acetaminophen concentration from the calibration graphs, previously prepared.

RESULTS AND DISCUSSION

Choice of the optimum conditions for combined polynomial method.

Following the general rules[5], the quadratic, p_2, and cubic, p_3, polynomials, have been chosen to construct the required combined polynomials, p_w for the determination of acetaminophen and phenacetin in presence of their degradation products. The quadratic polynomial, p_2, contributes greatly to the absorption curve of acetaminophen and/or phenacetin (Figs 2a&3a). The coefficients p_2 and p_3 have been calculated (Table 1) from the absorption spectra of each of the intact and the degraded drugs using six-, eight-, ten-, twelve- and fourteen-point orthogonal polynomials, each at 2-, 4-and 6-nm intervals. The signs and values of the integers a and b have been varied until p_w (z) is negligibly small relative to $\alpha_w C_x$. This requirement can be achieved by calculating the ratio $p_w (z)/p_w (X)$ which measures the expected percentage error in the determination of acetaminophen (or phenacetin)(X).

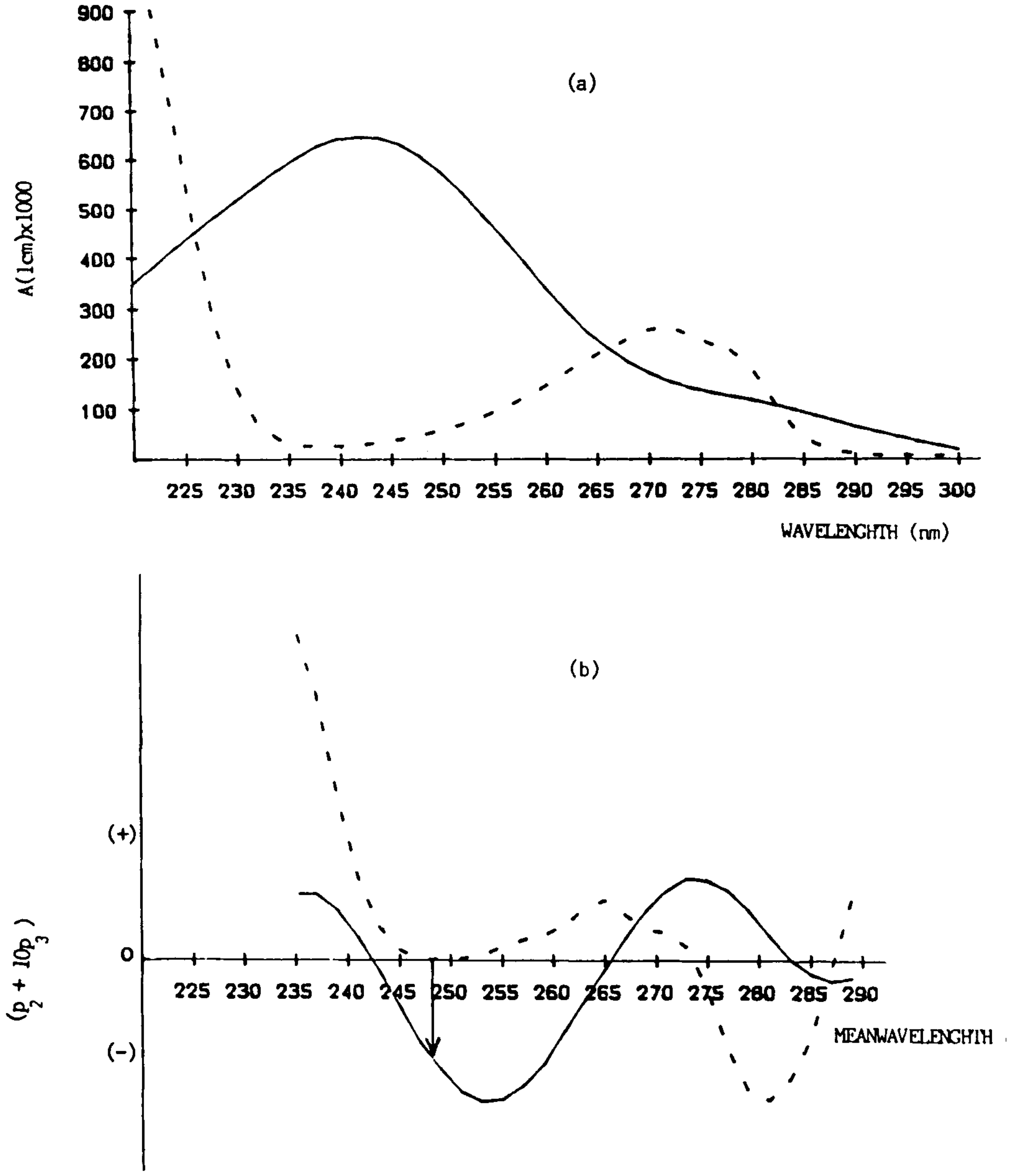

FIGURE 2

Absorption curves of 1 mg% w/v acetaminophen (——); its degradation product (---) in 0.1 N hydrochloric acid (a) and the corresponding convoluted curves derived therefrom using 12-points, (P_2+10P_3) orthogonal polynomials at 2 nm intervals (b).

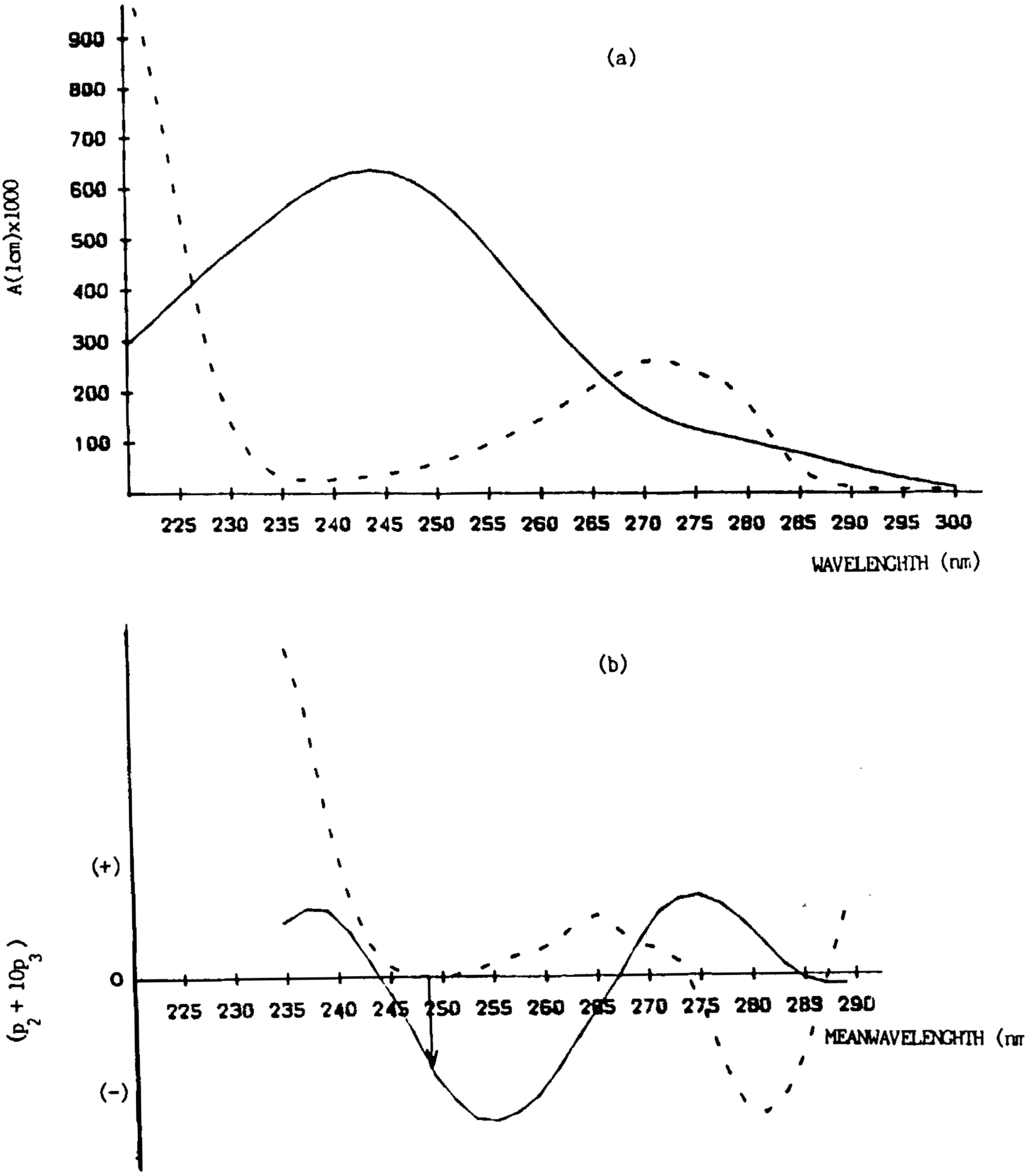

FIGURE 3

Absorption curves of 1 mg% w/v phenacetin (——); its degradation product (---) in 0.1 N hydrochloric acid (a) and corresponding convoluted curves derived therefrom using 12-points, (P_2+10P_3) orthogonal polynomials at 2 nm intervals (b).

TABLE 1

Sensitivity of $p_w=(p_2+10p_3)$ to 0.5-nm shift in the wavelength scale

Parameter	Compound, (X)					
	Acetaminophen			Phenacetin		
Wavelength, nm	$(\lambda_m - 0.5)$ 248.5	(λ_m) 249	$(\lambda_m + 0.5)$ 249.5	$(\lambda_m - 0.5)$ 248.5	(λ_m) 249	$(\lambda_m + 0.5)$ 249.5
Wavelength range (2 nm intervals)	237.5-259.5	238-260	238.5-260.5	237.5-259.5	238-260	238.5-260.5
$p_w(X).10^3$	-4.015	-4.272	-4.496	-3.416	-3.827	-4.089
$p_w(z).10^3$	0.043	0.041	0.046	0.041	0.040	0.045
Error (%)	1.071	0.960	1.023	1.200	1.045	1.101
Relative deviation (%) to p_w at λ_m	-6.016	0	+5.243	-10.739	0	+6.846

Such error is due to the presence of an equivalent concentration of its degradation products (z). In this connection, it was found that for $p_w = (p_2 + 10p_3)$ calculated over the wavelength range 238 to 260 nm at 2 nm intervals (λ_m=249 nm) and using twelve-point orthogonal polynomials, the expected percentage error for acetaminophen and phenacetin were 0.960% and 1.45% respectively (Table 1). Accordingly, the p_w-convoluted curves for the analysed compounds and their degraded products have been plotted (Figs 2b&3b). The finally chosen wavelength set and integers (a = 1 & b = 10) was occured on the slope of the drug p_w-convoluted curve. This means that the calculated p_w will be prone to the overall shifts in the spectrophotometer's wavelength scale. For that reason, an experiment has been designed to estimate the effect of ± 0.5 nm shift in the wavelength scale upon the p_w, calculated for acetaminophen and/or phenacetin. Thus, a solution of suitable drug concentration in 0.1 N hydrochloric acid was measured at three sets of wavelengths with (λ_m-0.5 nm); λ_m; (λ_m + 0.5 nm), where λ_m = 249 nm. Then, $p_w = (p_2 + 10p_3)$ was calculated for each set of twelve absorbances. The sensitivity of p_w at λ_m = 249 nm for ± 0.5 nm shift is shown in Table 1. To minimuze such effect the standard and sample should be measured side by side at constant temperature.

Having arrived at the finally chosen polynomials, wavelength range, intervals and values of a and b, the combined polynomials can be constructed.

Calibration curves and Reproducibility:

Under the above described parameters the graphs obtained by plotting p_w VS concentration (C,mg%) are linear within the range of 0.6-1.4 mg% for both drugs. The regression coefficient[11] was calculated to be 0.999 for both curves. The corresponding regression equations are:

$$p_w \times 10^3 = -0.056-4.249C \text{ (for acetaminophen)} \quad (4)$$

$$p_w \times 10^3 = 0.024-3.698C \text{ (for phenacetin)} \quad (5)$$

To assess the reproducibility of the proposed methods separate measurements of p_w were made for different concentra-

TABLE 2

Assay Results of Acetaminophen and Phenacetin in the Presence of their Degradation Products Using the Combined Polynomial (p_w), Orthgonal Function (P_2), and absorbance (A_{max}) methods.

Sample* Added (mg %)	Recovery (%)					
	Acetaminophen			Phenacetin		
	p_w	p_2	A_{max}	p_w	p_2	A_{max}
0.4	100.3	62.5	109.5	101.8	64.0	108.1
0.5	99.9	65.0	108.4	101.2	68.2	107.8
0.6	100.2	73.8	105.4	100.0	74.9	105.9
0.8	101.9	83.2	102.2	100.0	84.3	104.5
1.0	99.3	85.7	102.7	100.9	87.4	101.6
1.2	100.9	87.6	101.4	99.3	90.0	101.4
Mean	100.43	76.30	104.93	100.54	78.15	104.89
± SD	0.89	10.82	3.40	0.92	10.70	2.93

* Each contained either p-aminophenol (in acetaminophen samples) or p-phenetidine (in phenacetin samples), corresponding to 1 mg% of its intact.

tions of authentic acetaminophen/phenacetin in 0.1 N hydrochloric acid. The coefficient, p_w (1%, 1 cm) was calculated for each solution. The coefficient of variation was less than 2% indicating reasonable reproducibility.

Mixture Assay:

The applicability of the proposed methods was appraised through the determination of the intact drug in coexistence with the corresponding degradation product (Table 2). The results of the p_w were compared with those of A_{max} method and orthogonal function method (OFM). Applying OFM (using the quadratic polynomial, p_2, from the twelve-point orthogonal polynomials at 2 nm intervals, λ_m = 249 nm) the mean percentage

TABLE 3

Assay results for acetaminophen before (X) and after (Y) addition of p-aminophenol using P_w and A_{max} methods

Preparation	Mean[a] ± S.D.	
	P_w	A_{max}
Laboratory made Mixture[b]	100.43±0.89	104.93±3.40
Paracetamol Tablets		
(X)	100.85±0.46	100.55±1.78
(Y)	101.50±1.01	105.98±6.00
Paracetamol Syrup		
(X)	97.84±1.27	102.27±0.64
(Y)	97.69±0.84	105.91±0.99
Grippo Suppositories		
(X)	91.43±0.79	88.99±1.26
(Y)	89.97±1.32	95.00±2.53
Noflu Tablets		
(X)	100.58±0.67	101.68±1.05
(Y)	100.60±0.60	104.12±2.05

a- Mean value for 6 experiments (Lab. made mixture) or for 5 experiments (Pharmaceutical preparations).

b- Containing acetaminophen in concentration range of 0.4-1.2 mg% to which 1 mg% p-aminophenol was added

X Solutions containing 1 mg% acetaminophen

Y Solutions X to which 1 mg% p-aminophenol was added.

recoveries were equal to 76.30 ± 10.82 for acetaminophen and 78.15 ± 10.70 for phenacetin (Table 2). Such low results were mostly attributed to the contribution to p_2 from the degradation product which was cancelled by the p_w method. On the other hand, the high percentage recoveries calculated using A_{max} method were proportional to the degree of the irrelevant absorbance contribution from the degradation product. The latter was kept constant in variable drug concentration (Table 2).

Selectivity of the combined polynomial method:

The possible different intermediate products cannot be revealed by the above recovery experiments. Accordingly, stability investigations using the combined polynomial method have been carried out. Graphs of log C% (of the intact) vs time at different temperatures were linear(r=0.9954, 0.9998, and 0.9877 (for acetaminophen) and 0.9705, 0.9967 and 0.9888 (for phenacetin) at 50^{o}, 70^{o}, and 90^{o}C respectively). It may therefore be concluded that the hydrolysis rate of both compounds in strong acid medium (6 N hydrochloric acid) is first-order and the accuracy of the p_w method is not affected by the degradation products concentration.

Assay of acetaminophen pharmaceutical preparations:

Commercial preparations (tablets, syrup and suppositories) of acetaminophen in single dosage forms and combined with chlorpheniramine maleate and phenylpropanolamine hydrochloride (Noflu tablets) were assayed. Such an assay was conducted before and after p-aminophenol addition (Table 3). The results of p_w were compared with those of A_{max} method. The former method gave accurate results. Meanwhile A_{max} method gave high results due to p-aminophenol contribution. Besides their high accuracy and reproducibility the proposed method could be directly applied for acetaminophen assay even in the coexistence of its degradation product.

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