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Derivative spectrophotometric assay of acetaminophen and spectrofluorimetric determination of its main impurity*

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Abstract: Second derivative UV spectrophotometry proved to be a useful tool for the determination of acetaminophen, even in the presence of high amounts of the impurity, 4-aminophenol and the background effect caused by dissolved excipients of some dosage forms. The fluorimetric method used for the estimation of 4-aminophenol impurity is less sensitive than the colorimetric methods used in the pharmacopeias. This can be attributed to an inner-filter effect.

Keywords: *Acetaminophen; derivative spectrophotometry; fluorimetry; inner-filter effect.*

Introduction

Acetaminophen (paracetamol) is official in the United States [1] and in the British [2], European [3], German [4], and Japanese [5] pharmacopeias and is widely used as an analgesic and antipyretic agent [6]. Dry, pure acetaminophen is very stable at least up to a temperature not exceeding 45°C. However, if contaminated with traces of 4-aminophenol or exposed to humid conditions, hydrolysis to 4-aminophenol takes place, then further oxidative degradation may occur which can be characterized by a gradual colour change from pink to brown. This involves the breakdown of 4-aminophenol to quinoneimine and related compounds [7]. The degradation of acetaminophen in aqueous solution appears to be both acid and base catalysed reaction [8, 9]. The degradation kinetics are first order with respect to acetaminophen, the hydrogen ion and the hydroxide ion concentration [9]. The pharmacopeial limits for 4-aminophenol and 4-chloroacetanilide are 0.005 and 0.001% (w/w), respectively [1–5].

The aim of this work was to examine the possibility of a UV spectrophotometric method for acetaminophen in the presence of its main organic impurity, 4-aminophenol and also to study the fluorimetric determination of this impurity which can be present in the bulk substance and in some dosage forms both as an intermediate and as a degradation product.

Electroanalytical [10–12], gravimetric [13], titrimetric [14–16] and colorimetric [17–29] methods for acetaminophen are available. An HPTLC method is described for the determination of components containing acetaminophen in mixtures [30]. An ion-pair HPLC method has been described for the stability indicating assay of acetaminophen in effervescent tablets [31], another HPLC method determined acetaminophen in mixtures [32, 33]. As acetaminophen is often given with hydrocodone bitartarate in tablet formulations, three papers have addressed the analysis of this combination [34–36]. Other HPLC applications include simultaneous determination of acetaminophen and common pharmaceuticals in cough mixtures [37], determination in plasma [38], the simultaneous determination of acetaminophen, aspirin and caffeine [39], tablets analysis [40], the determination in urine [41], absorption and bioavailability of acetaminophen from hard gelation capsules [42], the simultaneous determination of propyphenazone, acetaminophen and caffeine in blood [43] and the determination of acetaminophen from serum used also HPLC but with electrochemical detection [44].

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Difference spectroscopy has been used for the determination of acetaminophen in an analgesic capsule preparation [45] and the simultaneous spectrophotometric determination of mefenamic acid and acetaminophen in 0.01 M hydrochloric acid in methanol was described [46]. The simultaneous determination of chlorzoxazone and acetaminophen in combined dosage forms was performed by an absorbance ratio technique and difference spectrophotometry [47]. Diode array spectroscopy was used for the analysis of acetaminophen/codeine phosphate combination tablets [48]. A computer assisted spectrophotometric method was developed for the assay of acetaminophen and phenacetin in the presence of their degradation products using combined orthogonal polynomials [49], and another method described the determination of acetaminophen and phenylpropanolamine hydrochloride in capsules by first derivative spectrophotometry [50].

The pharmacopeias [1–4] describe procedures for the determination of 4-aminophenol in acetaminophen colorimetric limit tests using sodium nitroprusside reagent, while [5] the phenol-hypobromite reaction. Some other colorimetric methods are also available [29, 30]. To study the applicability of our proposed fluorimetric method for the estimation of 4-aminophenol impurity, a series of literature data on the inner-filter effect was surveyed [51–59].

Experimental

Apparatus and reagents

Perkin–Elmer Lambda 5 and Lambda 15 recording spectrophotometers with 1 cm matched silica cells and a Hitachi MPF 2A recording spectrofluorimeter were used. The Lambda 5 spectrophotometer was equipped with a Data Station 3600. The 96% ethanol used for the spectrophotometric measurements was of spectroscopic grade (Merck Darmstadt, Germany), and the 0.1 N hydrochloric acid was prepared from hydrochloric acid of analytical grade. Methanol used for spectrofluorimetric measurements was of fluorescence spectroscopic grade (Merck Darmstadt, Germany). The quality of the reagents described in the pharmacopeial monographs fulfilled the requirements of the pharmacopeias.

Sample preparation

Acetaminophen and 4-aminophenol were dissolved in a suitable solvent. The ultraviolet absorption spectra of acetaminophen and 4-aminophenol in ethanol and in 0.1 N hydrochloric acid in the same concentration are shown in Figs 1 and 2. Tablets, including effervescent tablets were reduced to a fine powder, then the active ingredient content was

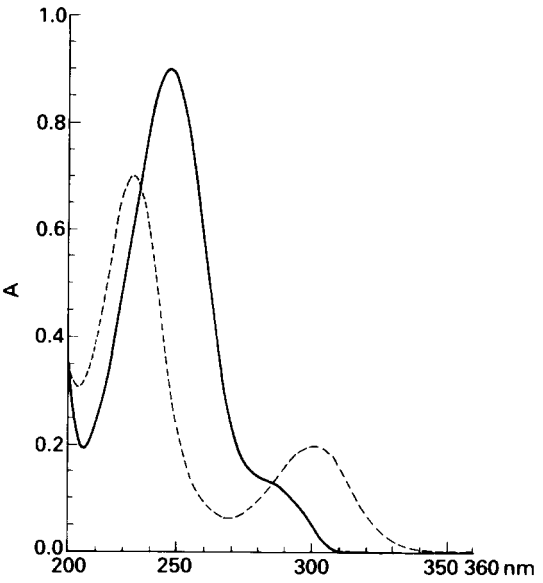

Figure 1
Ultraviolet absorption spectra of 10 μg ml^{-1} acetaminophen (——) and 10 μg ml^{-1} 4-aminophenol (·····) in ethanol.

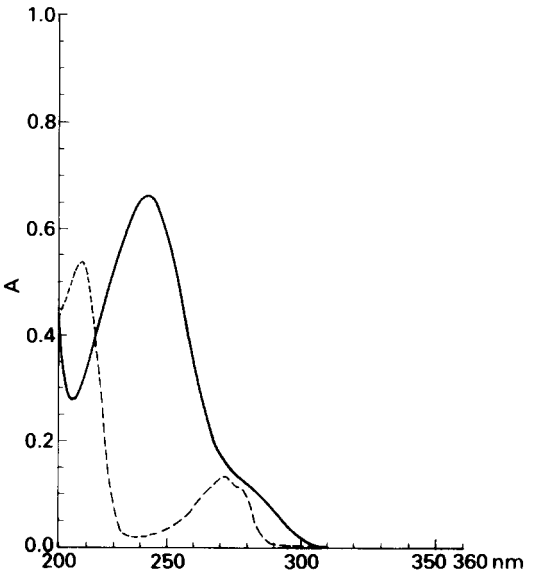

Figure 2
Ultraviolet absorption spectra of 10 μg ml^{-1} acetaminophen (——) and 10 μg ml^{-1} 4-aminophenol (·····) in 0.1 N HCl.

quickly dissolved by shaking it either with ethanol, or with 0.1 N hydrochloric acid. A significant background effect caused by the dissolved excipient(s) was observed in the case of effervescent tablets on the short wavelength slope of the main band. This was eliminated using the well-known base-line correction method first used by Morton and Stubbs (60). The analytical range of concentrations was 0.005–0.040 mg ml^{-1}. The evaluation of the second derivative spectra was based on peak to trough measurements. Displaying the positive and negative peak values to three decimal places enabled significantly more accurate evaluation of the amplitudes than a conventional method measuring the distance between peak and trough manually. The amplitude calibration graphs were found to be linear passing through the origin, (r = 0.999).

The second derivative scanning conditions on a Perkin–Elmer Lambda 5 spectrophotometer were: scan speed: 60 nm min^{-1}; spectral slit width: 1 nm; response time: 0.5 s. The fluorimetric method based on the native fluorescence of 4-aminophenol was less sensitive than the colorimetric methods of the previously mentioned pharmacopeias [1–5], but the inner-filter effect of the 2000 fold excess acetaminophen results in a positive intercept in the calibration curve of 4-aminophenol. Fluorescence was measured in methanol at the following wavelengths: λ_{exc} = 308 nm, λ_{em} = 370 nm.

Results and Discussion

The UV spectra of acetaminophen were recorded in ethanol, methanol, 0.1 N hydrochloric acid in ethanol, water, 0.01 N sodium hydroxide. These spectra were different, but all showed two bands. The short wavelength intense band corresponds to a transition (^1L_a band), while the long wavelength band of lower intensity occurs generally only as an inflexion on the long wavelength slope of the main band and can be assigned according to the nomenclature of Klevens and Platt as a $^1L_b \leftarrow {}^1A$ band. The maxima of the main band was found to be dependent on the solvent in the 240 nm (in 0.1 N hydrochloric acid)–258 nm (in 0.01 N sodium hydroxide) range. The bathochromic shift in alkaline media was attributed to phenolate formation [4, 61, 62].

4-Aminophenol is also a p-disubstituted benzene derivative. Dependent on the substituents, significant bathochromic shifts occur in the spectra of p-disubstituted benzene derivatives related to that of benzene [63], this phenomenon can be observed in the UV spectra of 4-aminophenol in ethanol and also 0.1 N hydrochloric acid [64]. The fact that the UV absorption bands of acetaminophen and of 4-aminophenol exhibit more or less overlapping bands, may cause difficulties in the UV spectrophotometric determination of acetaminophen only if much more than the allowable amount of impurity would be present. Less than 1% of 4-aminophenol did not cause a significant error. As mentioned above, acetaminophen is a rather stable compound. However, if not stored correctly or incorporated in a less suitable dosage form, the amount of impurity may increase. The decomposition pathway is shown in Fig. 3. In our experiments derivative spectrophotometry proved to be a useful method to eliminate any disturbing effect of even higher amounts of 4-aminophenol. Acetaminophen is used in relatively high doses (0.50 g per tablet), its molar absorbance is rather high, dependent on the solvent the value of ϵ is 9500–13000, so it is unlikely that a background effect caused by dissolved excipients should occur. But it is conceivable that in some dosage forms, for example in effervescent tablets, the active ingredient: excipient ratio is lower and a background effect may occur.

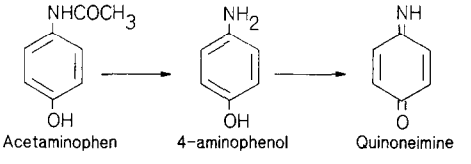

Figure 3
Degradation of acetaminophen.

Second derivative spectrophotometry eliminated both the background effect caused by dissolved excipients and the interference of higher amounts of 4-aminophenol. The second derivative spectra of acetamino-4-phen in the presence of 1% 4-aminophenol are shown in Figs 4 and 5.

Most methods suitable for determining 4-aminophenol as an impurity in acetaminophen involve spectrophotometric measurement of a chromophore prepared with a suitable reagent, or combined with chromatographic separation. Earlier literature data were available on the fluorescence of acetaminophen in different

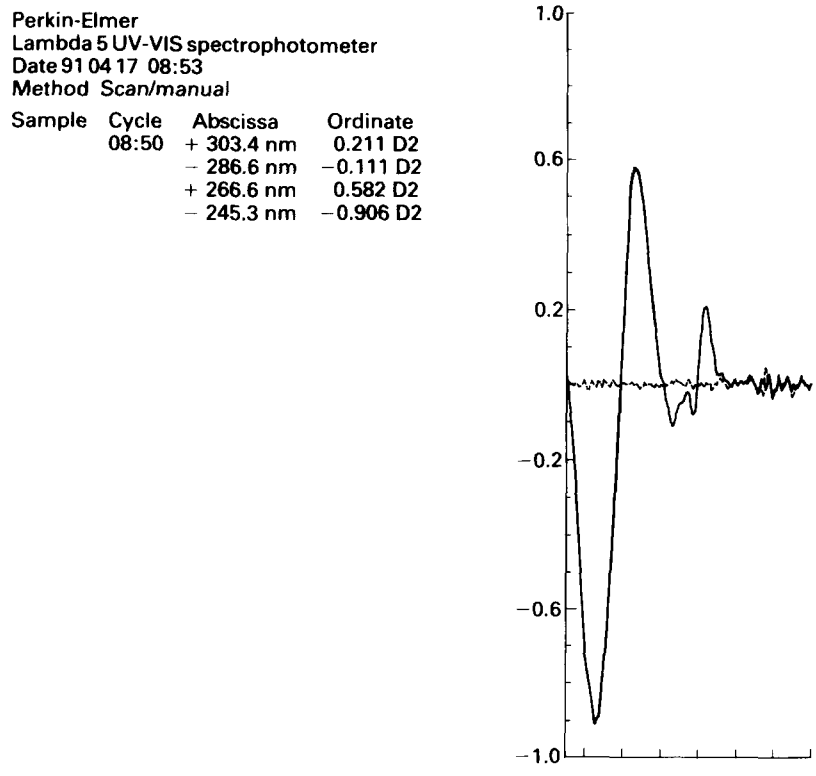

Figure 4
Second derivative spectra of 10 μg ml^{-1} acetaminophen (——) in ethanol and in the presence of 0.1 μg ml^{-1} 4-aminophenol (1%) (·····).

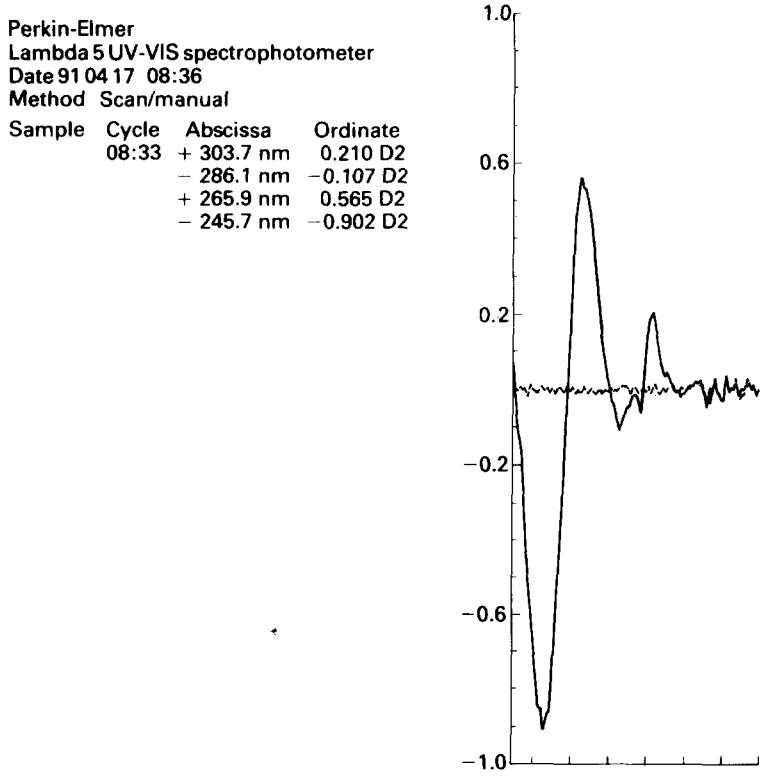

Figure 5
Second derivative spectra of 10 μg ml^{-1} acetaminophen (——) in 0.1 N HCl and in the presence of 0.1 μg ml^{-1} 4-aminophenol (1%) (·····).

Table 1
Summary of assay results comparing three pharmacoepeial methods with the methods for acetaminophen and 4-aminophenol described in this work

		USP XXII		BP.88 Ph. Eur.II DAB 9		Ph.Jap.XI		Present method	
Samples	Requirements	Impurity* ≤0.005%	Assay 98.0–101.0%	Impurity* ≤0.005%	Assay 99.0–101.0%	Impurity* ≤0.005%	Assay ≧98.0%	Impurity* —	Assay 99.0–101%
Acetaminophen bulk substance I		<0.005%	99.5%	<0.005%	99.5%	<0.005%	99.0%	0.05% (limit of sensitivity)	99.7% $n = 7$ RSD = 1.1%
Acetaminophen bulk substance II		<0.005%	99.2%	<0.005%	99.3%	<0.005%	99.0%	0.05% (limit of sensitivity)	99.5% $n = 7$ RSD = 1.0%
Acetaminophen tablets†	Found values	<0.005%	0.500 g	<0.005%	0.495 g	—	—	0.05% (limit of sensitivity)	0.498 g $n = 7$ RSD = 1.3%
Acetaminophen effervescent tablets I†		<0.005%	0.495 g	<0.005%	0.490 g	—	—	0.05% (limit of sensitivity)	0.493 g $n = 7$ RSD = 1.6%
Acetaminophen effervescent tablets II† (expired formulation)		0.5%	0.485 g	0.5%	0.490 g	—	—	0.5%	0.475 g $n = 7$ RSD = 2.3%

*4-aminophenol impurity,
†Calculated with reference to the average mass.

aqueous solvents [65]; however, recent experiments could not confirm these earlier findings which probably could be attributed to Raman emission of water and to poorly aligned monochromator systems. Acetanilide is not fluorescent but the introduction of a *p*-hydroxyl-group into the molecule induces fluorescence [66]. Neither exhibits fluorescent 4-chloroacetanilide. Indirect fluorimetric methods suitable for the determination of acetaminophen can be found in the literature [67, 68], but these do not influence the fluorimetric detection and determination of 4-aminophenol.

Fluorescence spectrometry is one of the most sensitive techniques for the determination of minute amounts in samples. Unfortunately, numerous interferences may occur, such as quenching of the fluorescence of the sample by other matrix components [51]. If the fluorescence experiment involves the addition of an absorbing molecule to the fluorescent species, a decrease in the fluorescence intensity due to the absorption of light by the added molecule may result [55]. This absorption was first described by Parker as the inner-filter effect [56]. The inner-filter effect may involve one or two components depending on the spectral properties of the absorbing species. Primary absorption occurs when the species absorbs light at the wavelength of excitation, which decreases the amount of light available to excite the fluorophore. For quantitative analytical purposes the inner-filter effect must be either avoided or compensated. The inner-filter effect can be avoided by diluting the species involved, but this is not always possible. An available method makes use of a 'blank' solution to correct for the effect of the absorbing species. This method was also used in our method. The fluorescence emission spectra of 10 μg ml^{-1} acetaminophen in methanol in the presence of different amounts of 4-aminophenol are shown in Fig. 6.

Table 1 summarizes the results of the second derivative spectrophotometric and spectrofluorimetric methods. It must be noted that the assay methods of the British, European and German pharmacopeias agree; the 4-aminophenol impurity test methods and requirements of the United States, British, European and German pharmacopeias agree as well. The conclusion can be drawn, that second derivation UV spectrophotometry proved to be very useful for the determination of acetaminophen even in the presence of higher amounts of 4-aminophenol impurity and/or in the presence of background effect caused by dissolved excipients. The fluorimetric method used for the estimation of 4-aminophenol impurity is less sensitive than the colorimetric methods used in the pharmacopeias.

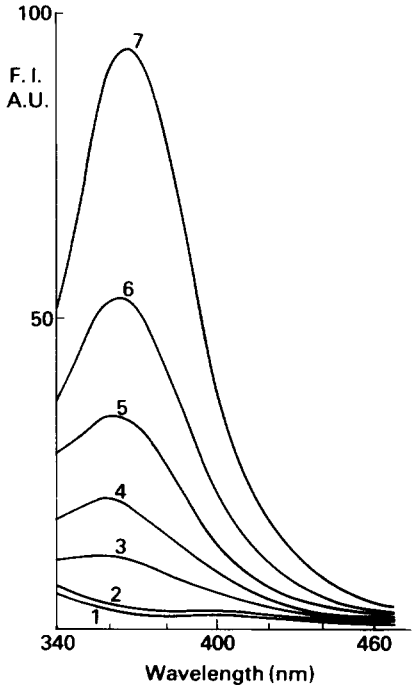

Figure 6
Fluorescence emission spectra of 10 μg ml^{-1} acetaminophen in methanol and in the presence of various amounts of 4-aminophenol. (1) blank; (2) 10 μg ml^{-1} acetaminophen; (3) 10 μg ml^{-1} acetaminophen +0.05% 4-aminophenol; (4) 10 μg ml^{-1} acetaminophen +0.1% 4-aminophenol; (5) 10 μg ml^{-1} acetaminophen +0.3% 4-aminophenol; (6) 10 μg ml^{-1} acetaminophen +0.5% 4-aminophenol; (7) 10 μg ml^{-1} acetaminophen +1.0% 4-aminophenol.

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