ANALYSIS OF EFERALGAN TABLETS BY FIRST-ORDER DERIVATIVE UV-SPECTROPHOTOMETRY

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A simple analytical method is described for the simultaneous determination of acetaminophen (AAP) and ascorbic acid (ASA) in effervescent tablets of Eferalgan. The first-order derivative UV-spectrophotometry is employed. By working in phosphate buffer medium (*p*H=7.0), the zero-crossing wavelengths are found at 243.5 and 264.5 nm for AAP and ASA, respectively. The calibration graphs are rectilinear up to $1.4 \cdot 10^{-4}$ M AAP and $1.6 \cdot 10^{-4}$ M ASA and the limits of detection are $1.7 \cdot 10^{-6}$ M AAP and $2.0 \cdot 10^{-6}$ M ASA, respectively. RSD values lower to 3 % were found for $4.25 \cdot 10^{-5}$ M AAP and for $5.16 \cdot 10^{-5}$ M ASA, respectively.

Introduction

Acetaminophen (AAP - N-acetyl-p-aminophenol, also known as Paracetamol) and L-ascorbic acid (ASA - vitamin C) are two organic compounds with important biological activities. Thus, AAP is widely used as analgesic and antipyretic drug [1] while ASA is a hydrosoluble vitamin essential for the health of human beings. In biochemical processes, ASA can play roles of substrate, carrier of hydrogen and cofactor and, owing to its antioxidant properties, is considered to have an anticarcinogenic activity [2].

In the recent past, efficient analytical methods have been developed in our University for individual determinations of AAP [3] or ASA [4,5] by flow injection with biamperometric [3,5] or spectrophotometric [4] detection. Those methods were applied in pharmaceutical [3] or natural juices [4,5] analysis. Nevertheless, they are not appropriate to simultaneously determine the two substances from their mixed-together dosage forms.

ASA and AAP are formulated together in pharmaceutical tablets administrated for pain and fever and commercialised on Romanian market under the name of Eferalgan. To analyse products of this kind, containing two or more compounds with pharmaceutical activity, some variants of multicomponent analysis have to be used. A good possibility is represented by HPLC but it needs expensive apparatus and involves complex working procedures. However, this analysis way was applied to discriminate the AAP from its degradation product in aged pharmaceuticals [6]. Another possibility is to take spectrophotometric measurements at more wavelengths and to perform then multivariate regressions [7,8]. In this case, the calculus is complicated and requires extensive off-line processing of data and specialised software. Recently, such an analytical method has been proposed to analyse mixtures of AAP and acetylsalicylic acid [9].

A more convenient alternative of multicomponent analysis is the employment of the derivative spectrophotometry [10], that greatly improves the determination selectivity. It

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was used to resolve AAP from mixtures with orphenadrine citrate, ibuprofen or chlorzoxazone [11], with caffeine and acetylsalicylic acid [12] and with only caffeine [13] while ASA was resolved from mixtures with menadione [14].

Normally, the derivative spectra are computed mathematically by the Savitzky-Golay method [15], implemented now on the new generation of spectrophotometers, or by original computer programmes [16]. The optical or electronic acquirement of derivative spectra is also described in the literature [17], but rarely used in the experimental practice. However, the zero-crossing technique [10] is almost always employed for data processing, independent on the manner of acquiring the derivative spectra.

In this paper, the simultaneous determination of AAP and ASA in Eferalgan tablets is presented. It is performed by first-order derivative UV-spectrophotometry employing spectra computed mathematically by the spectrophotometer software. Although the absorption bands of ASA and AAP overlap significantly, good results are obtained by the zero-crossing technique.

Experimental

Reagents of analytical grade were employed throughout and all solutions were prepared in distilled water. The used AAP and ASA were Fluka products. KH_2PO_4 (Fluka) and Na_2HPO_4 (Reactivul) were used to prepare a stock solution of phosphate buffer (*p*H=7.0) according to the reference [18]. Effervescent tablets of Eferalgan (UPSA Laboratoires, France) were purchased from commerce. Their claimed composition is: (330 mg AAP, 200 mg ASA and 2,420 mg unspecified water-soluble excipients)/tablet.

Stock solutions of ASA and AAP, respectively, were prepared initially at milimolars concentrations. From these solutions, intermediate working solutions were obtained by 10-fold dilution. The measured solutions of ASA or AAP were prepared in 25 mL volumetric flasks from 2.5 mL stock solution of buffer, appropriate volumes of intermediate working solutions and water to volume.

Five different tablets of Eferalgan were individually analysed. Each tablet was solved separately in water and the obtained solution was quantitatively passed in an 1 L volumetric flask. From the solutions obtained so, 7.5 mL were pipetted in 100 mL volumetric flask where 10 mL stock solution of buffer and water to volume were also added. The amounts of ASA and AAP, respectively, were calculated using the derivative spectra and the calibration curves prepared previously at the selected zero-crossing wavelengths.

The absorption spectra were recorded over the range 200÷320 nm with the spectrophotometer Jasco (Japan), Model V-530, at maximum 30 min after solution preparations. The scanning rate and spectral window were of 400 nm/min and 2 nm, respectively. Quartz cells with 1.000 cm optical path were employed. The reference solutions were similarly prepared to the measured solutions, but they contained only the buffer. The first-order derivative spectra were computed mathematically by the “Spectra Analysis” software of the employed instrument using a data window of five points. The values were calculated and reported at wavelengths spaced by 1 nm.

The titrimetry was adopted as method of reference. To determine ASA, a known amount of iodine was added over acidified solutions obtained from the analysed pharmaceuticals. The excess iodine was titrated with standardised thiosulphate solution in the presence of soluble starch as indicator. This procedure is the non-automated version of the methods proposed previously [4,5]. The sum of ASA and AAP was determined on a different sample solution by titration with bromate in the presence of excess bromide and methyl red indicator, as described elsewhere [19].

Results and discussions

In a preliminary step, a survey has been performed regarding the absorption spectra dependence on *p*H for each of the studied analytes. In this respect, absorption spectra were recorded for solutions prepared in 0.1 M HCl; phthalate (*p*H=3.0)-, acetate (*p*H=5.0)-, phosphate (*p*H=7.0)-, ammonia (*p*H=9.0)- and borate (*p*H=11.0) buffers; and 0.1 M KOH, respectively. In alkaline solutions, the ASA was found to be unstable while the absorption spectra recorded for acidic or neutral solutions of each analyte remained unchanged for two hours (maximum investigated). Consequently, only neutral or acidic solutions are appropriate for measurements and were considered in the further work.

Both analytes absorb under 300 nm, the shape of the spectra depending strongly on the *p*H. Moreover, the absorption bands of the two compounds are highly overlapped. In solutions of 0.1 M HCl, AAP and ASA have the absorption maxims located at 230 and 244 nm, respectively, they being separated by only 14 nm. Increasing the *p*H, the absorption maxima move towards greater wavelengths for both analytes and the difference between them increases. The maximum separation between the absorption bands of the two analytes was found at *p*H=7.0. Consequently, only phosphate buffer was employed as chemical medium in the subsequent work.

In Fig. 1, the zero-order absorption spectra are presented comparatively for solutions of AAP 6.34×10^{-5} M, ASA 5.76×10^{-5} M and Eferalgan 221 μg/mL, respectively. The last solution was obtained as described in the Experimental section from an Eferalgan tablet with the weight of 2.9447 g. A significant overlap still exist between the absorption bands of AAP and ASA even at the working *p*H selected as optimum. Thus, the absorption maxims are now situated at 244 nm for AAP (ε=100,000 $L \cdot mole^{-1} \cdot cm^{-1}$) and at 265 nm for ASA (ε=110,080 $L \cdot mole^{-1} \cdot cm^{-1}$), respectively. At the same time, the absorption bands of AAP and ASA are not distinguished in the absorption spectrum of the Eferalgan solution. As can be seen, this spectrum is very similar with the spectra corresponding to the pure organic compounds that present a single, broad absorption band.

The first-order derivative spectra calculated by the instrument computer from the spectra shown in Fig. 1 are presented in Fig. 2. For AAP, the derivative spectrum crosses the abscise between 243 and 244 nm, respectively. At the mentioned wavelengths, the derivative absorbance has values of opposite sign nearly identical in magnitude. Consequently, the wavelength of zero-crossing is considered to be λ_1=243.5 nm. Similarly, the wavelength of zero-crossing in the derivative spectrum of ASA is considered λ_2=264.5 nm.

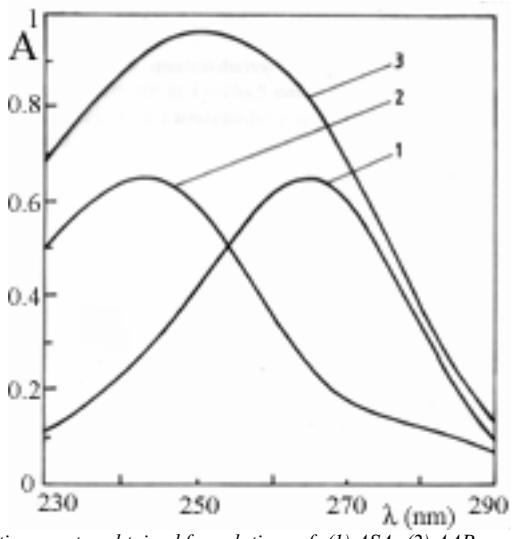

Fig. 1: *Absorption spectra obtained for solutions of: (1) ASA; (2) AAP; and (3) Eferalgan, respectively. See text.*

Because the excipients used normally in the pharmaceutical products together with the active substances do not absorb at wavelengths greater than 200 nm, the signal values found in the first-order derivative spectra of the Eferalgan solutions at the wavelengths of zero-crossing λ_1 and λ_2 would have to be suitable for determinations of ASA and AAP, respectively. Consequently, calibration graphs were prepared by working with standard solutions containing only one of the two analytes at a time. The following equations were obtained so:

$$D(A)_1 = (+0.0001 \pm 0.0002) + (269 \pm 3) \times C_{ASA} \quad (1)$$

$$D(A)_2 = (-0.0001 \pm 0.0003) + (300 \pm 3) \times C_{AAP} \quad (2)$$

where $D(A)_1$ and $D(A)_2$ are the values of the derivative absorbance at 243.5 and 264.5 nm, respectively, and C_{ASA}, C_{AAP} are the concentrations of ASA or AAP, expressed in mole·L^{-1}. $D(A)_1$ and $D(A)_2$ were obtained as averages of the signal values present in the derivative spectra at 243 and 244 nm (first case) and at 264 and 265 nm (second case), respectively.

The equations (1) and (2) apply up to concentrations of $1.6 \cdot 10^{-4}$ M ASA or $1.4 \cdot 10^{-4}$ M AAP, respectively. The detection limits are $2.0 \cdot 10^{-6}$ M ASA and $1.7 \cdot 10^{-6}$ M AAP, respectively, and the found relative standard deviations were 2.4 % for $5.16 \cdot 10^{-5}$ M ASA (n=5) and 1.9 % for $4.25 \cdot 10^{-5}$ M AAP (n=5), respectively.

The results obtained in the analysis of the Eferalgan tablets are presented in Table 1 as average ± standard deviation of the amounts found in each of the five tablets, analysed by both spectrophotometry and titrimetry. The agreement between the two methods is good, in limits of ±2 %, and also with the label claim. It proves the excipients found in the analysed product do not interfere with the determinations, the spectrophotometric method proposed

here being applicable to dosage control for both AAP and ASA in Eferalgan. It also could be employed to perform uniformity tests.

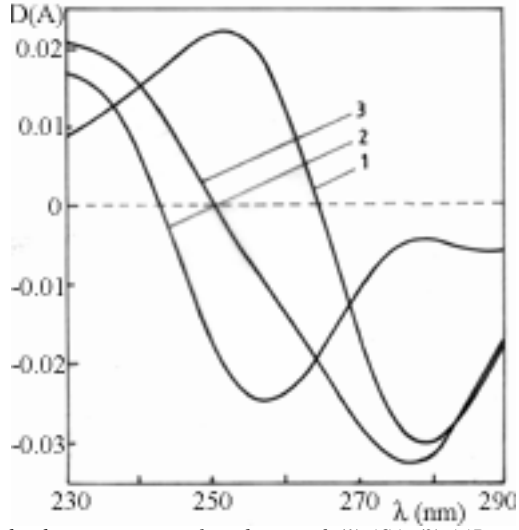

Fig. 2: *First-order derivative spectra for solutions of: (1) ASA; (2) AAP; and (3) Eferalgan, respectively. See text.*

A recovery test was performed for each analysed tablet by employing $3.18 \cdot 10^{-5}$ M AAP and $4.17 \cdot 10^{-5}$ M ASA added standards. The results obtained so are also presented in Table 1 similarly with the previous results. In statistical terms, the found values are identical with 100 % and proves that the analysis of the pharmaceutical product Eferalgan is free of matrix interferences.

Table 1. Results obtained in the analysis of Eferalgan tablets

Analyte	mg / tablet found by:		Difference (%)	Recovery (%) of
	New Method	Titrimetry	between methods	added standard
AAP	199 ± 5	202 ± 9	-1.5	98 ± 6
ASA	333 ± 8	328 ± 6	+1.5	103 ± 7

Conclusions

A simple and accurate method has been proposed for the simultaneous determination of AAP and ASA in effervescent tablets of Eferalgan. It is based on the employment of the first-order UV-spectrophotometry.

The measured solutions were buffered at *p*H=7.0 to obtain a good separation between the absorption bands of the analytes. In such conditions, the zero-crossing wavelengths are found in the first-order derivative spectra at 243.5- and 264.5 nm for AAP and ASA, respectively. For both investigated compounds, accurate determinations can be performed in the concentration ranges of 10^{-5} - 10^{-4} M, the limits of detection being close to 10^{-6} M.

The results obtained in the analysis of Eferalgan tablets agree well with those determined by titrimetric procedures. However, the proposed method has many advantages in comparison with titrimetry. It is rapid, eliminates the tedious titration operations, employs few chemical reagents and allows simultaneous determinations with similar precisions for both analytes.

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