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Study of the influence of differentiation parameters on the characteristics of derivative electronic absorption spectra

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The article presents the results of a study of the characteristics of first- to fourth-order derivatives of papaverine hydrochloride absorption spectra obtained using scanning spectrophotometer software. Using the example of an asymmetric shortwave absorption band, the transformation of zero-order spectra upon differentiation with various $\Delta\lambda$ intervals from 1 to 80 nm is graphically demonstrated. The influence of the specified parameters (scanning step and differentiation interval) on the shape of the derivatives is assessed. It is established that with increasing $\Delta\lambda$, the curve shapes, positions of extrema, and „zero crossings"change. The obtained results can be used to develop methods for the identification and quantification of individual compounds and the analysis of multicomponent systems. The use of the spectrophotometer software allows for the rapid and reliable selection of optimal scanning parameters and differentiation of absorption spectra.

Keywords: absorption spectra, differentiation, derivative spectrophotometry, papaverine hydrochloride.

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Introduction

Differentiation of electronic absorption spectra with respect to wavelength forms the basis of derivative spectrophotometry (DS), widely used in chemical analysis of environmental objects, pharmaceutical and food products, and biological systems [1–5]. Derivative spectrophotometry is one of the spectrophotometry (SP) methods in the UV and visible regions, holding a special place in pharmaceutical analysis for determining the qualitative and quantitative chemical composition of pharmacologically and biologically active substances [6–10]. SP is employed to assess the authenticity of pharmaceutical substances and quantify active ingredients in both substances and finished dosage forms. The method is included in the State Pharmacopoeia of the Russian Federation (SP RF) [11] and all national pharmacopoeias. Modern spectrophotometers integrated with computers and equipped with appropriate software enable rapid and accurate acquisition of spectral data. Recording electronic absorption spectra in the 190–1100 nm range using scanning spectrophotometers takes just tens of seconds, enhancing the speed of information acquisition. Automated graphics control significantly simplifies spectrum interpretation. Spectrophotometer software facilitates spectral data processing (addition, subtraction, multiplication of spectra, obtaining derivatives of various orders, integration, etc.), substantially expanding SP capabilities.

The analytical signal in DS is the nth-order derivative $d^nA/d\lambda^n$, while the graphical dependence $d^nA/d\lambda^n = f(\lambda)$ shows the resulting differential curve, which has a structure more clearly expressed than in the original absorption spectrum [12–14]. Currently, first- to fifth-order derivatives are most commonly used in practice, providing information on the structure, physicochemical properties, and reactivity of organic and inorganic compounds [15–17]. Application of higher-order derivatives is limited, as repeated differentiation substantially increases result errors. Derivative spectrophotometry is actively used in quantitative analysis, as linear dependence of the analytical signal on the concentration of the absorbing substance holds for derivative absorption spectra, and additivity of analytical signals is observed in systems with multiple absorbing components [18–22].

The ability of DS to simply and accessibly analyze complex multicomponent systems without additional separation procedures attracts researchers. Literature analysis reveals growing interest in applying DS to develop new and refine existing analytical methods. Their main advantages include the absence of labor-intensive sample preparation stages, reagent and time savings, waste minimization, equipment accessibility without specific operating conditions or highly qualified personnel. Another undeniable advantage of DS is its compliance with the principles of „green" analytical chemistry [23–25].

When developing methods using spectrophotometer software, it is essential to consider that instrumental parameters affect result reproducibility. It has been noted that initial absorption spectrum recording conditions strongly influence derivative shape. Differentiation can yield more pronounced and narrower bands or amplify noise signals [1]. Thus, selecting optimal differentiation conditions is crucial. However, corresponding original studies often lack justification for condition and differentiation order selection, preventing reproduction of proposed methods with other software. In some works, such data are simply absent.

Accordingly, the study's aim was to evaluate the capabilities of scanning spectrophotometer software for obtaining 1–4 order derivatives and investigate the influence of differentiation parameters on papaverine hydrochloride electronic absorption spectrum derivatives.

Materials and methods

Electronic absorption spectra were recorded on a double-beam scanning spectrophotometer SHIMADZU UV–1800 (Japan) in „spectral" mode. Optical density scanning of solutions versus wavelength at „fast" speed was performed at 0.1, 0.2, 0.5, and 1.0 nm intervals in the 200–400 nm range using quartz cuvettes ($l = 10$ mm). In all cases, spectral slit width was 1 nm. Photometric accuracy of the spectrophotometer was ± 0.002 and ± 0.004 at optical densities of 0.5 and 1.0, respectively. Wavelength scale reproducibility was ± 0.1 nm.

The spectrophotometer software UV-Probe 2.31 enables spectral data processing and various mathematical and graphical transformations of spectral curves, particularly calculating 1–4 th-order derivatives. Savitzky-Golay algorithm is used for derivative computation. The differentiation interval ($\Delta\lambda$) is determined by initial spectrum scanning parameters. At 0.1 nm scanning, software allows $d^nA/d\lambda^n$ of $\Delta\lambda = 1$, 2, 4 and 8 nm; at 0.2 nm scanning, $\Delta\lambda$ values are 2, 4, 8 and 16 nm; at 0.5 nm scanning, 5, 10, 20, and 40 nm; at 1.0 nm scanning, 10, 20, 40, and 80 nm. Software offers a derivative amplitude magnification option — a scaling factor set to 100.

Pharmaceutical substance papaverine hydrochloride (PH) with > 99% active ingredient content (Recordati Industria Chimica and Farmaceutica S.p.A., Italy) was used. Stock solution of 100 mg/l was prepared by dissolving an exact substance in 0.1 M solution of hydrochloric acid weigh on analytical balances „HR-200" („A&D", Japan, ± 0.0001 g). For constructing optical density (A) dependence on PH concentration (C, mg/l) a series of solutions was prepared by diluting the stock, ensuring A values fell within 0.2–1.2 range. Verification of Beer-Lambert law compliance and molar absorptivity determination followed methods in [26]. Linearity reliability $A = a + bC$ was confirmed by correlation analysis [27]. MS Excel software was used to process the data.

Experimental results and their discussion

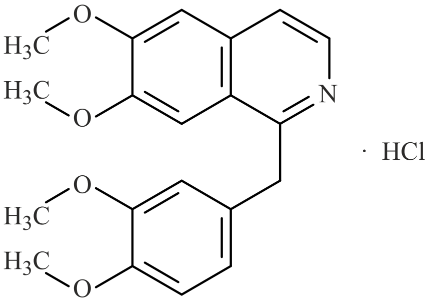

Figure 1. Structural formula of PH

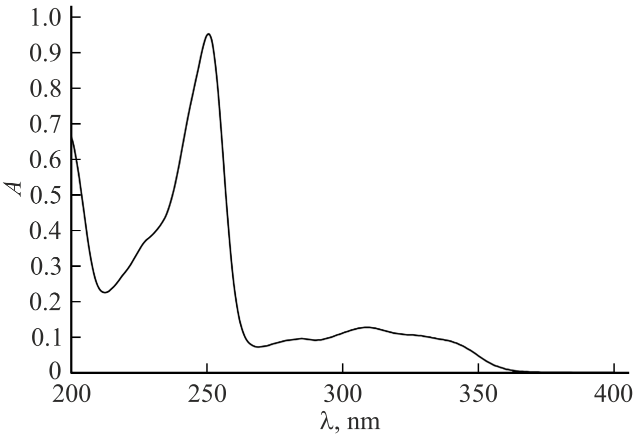

Figure 2. Electronic absorption spectra of PH hydrochloric acid solution: reference solution 0.1 M HCl, $C(\mathrm{PH}) = 6.0$ mg/l, scanning step 0.1 nm.

Papaverine hydrochloride — 6,7-dimethoxy-1-(3′,4′-dimethoxybenzyl)isoquinoline hydrochloride — is a white crystalline powder soluble in chloroform, moderately soluble in water, and slightly soluble in 96% ethanol. In medical practice, it serves as a spasmolytic agent included in various mono- and multicomponent dosage forms (tablets, suppositories, injection solutions) [28]. 0.1 M aqueous HCl was selected as solvent to suppress hydrolysis and stabilize the compound in solution. PH structural formula is shown in Fig. 1.

At the first research stage, spectral characteristics of PH hydrochloric acid solution absorption curve needed determination. The initial electronic absorption spectrum (zero-order) in 200–400 nm range shows two bands of different shape and intensity (Fig. 2). The high-intensity shortwave band with an arm in 220–234 nm, range, corresponding to allowed $\pi \to \pi^*$ transition, features absorption maximum ($\lambda_{\max}$) at 251 nm and minimum ($\lambda_{\min}$) at 213 nm. The broad longwave band (270–370 nm) corresponding to $n \to \pi^*$ transition, has low intensity, indistinct maxima (285 and 309 nm), minima (270 and 290 nm), and an arm in 320–345 nm range. Above 370 nm, the compound does not absorb. Electronic absorption spectra recorded at various intervals (0.1 to 1.0 nm) at „fast" scanning speed matched across all spectral characteristics.

Since any SP method application requires Beer-Lambert law compliance [12] concentration ranges were established where solution optical density versus PH concentration showed linear dependence. Linearity reliability was confirmed by correlation analysis [27]. and intensity differences cause linearity range variations, as absorption

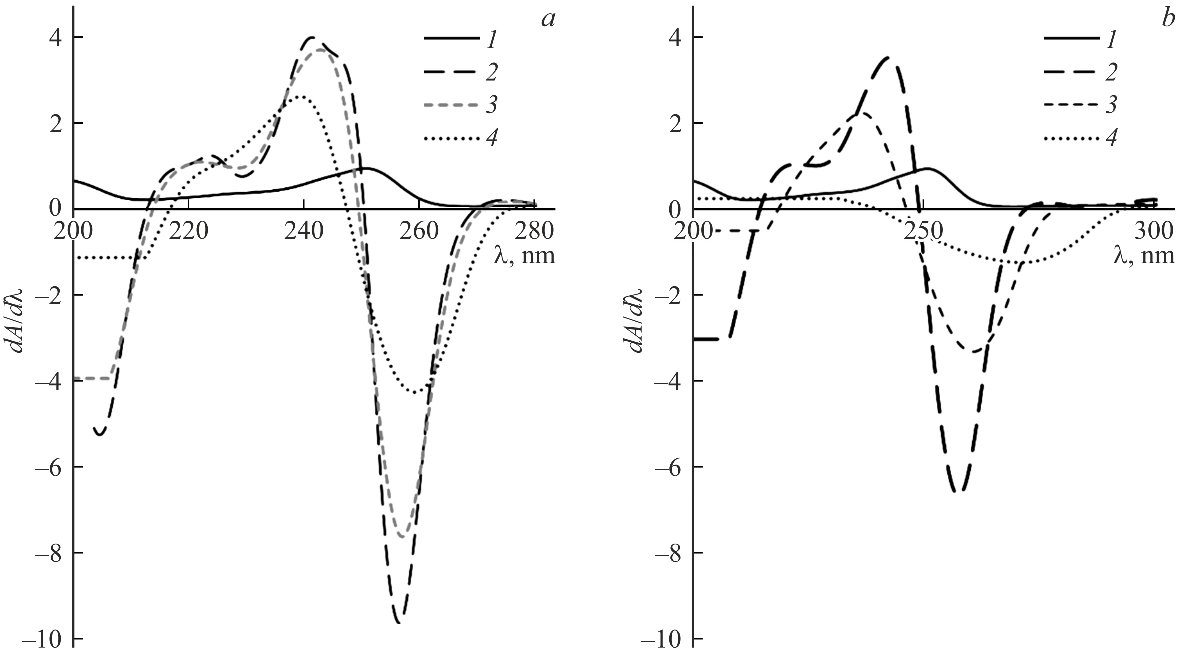

Figure 3. Papaverine hydrochloride absorption spectrum of zero order (*1*) and first derivatives obtained at various $\Delta\lambda$ values: (*a*) $\Delta\lambda = 4$ (*2*), 8 (*3*), 16 nm (*4*) (scanning step 0.2 nm); (*b*) $\Delta\lambda = 10$ (*2*), 20 (*3*), 40 nm (*4*) ((scanning step 0.5 nm).

measurement error is minimal at solution optical densities from 0.2 to 1.2 [13]. Calculated molar extinction coefficients were 60100 (l/mol · cm) at wavelength of 251 nm, 6400 and 8300 (l/mol · cm) at 285 and 309 nm respectively. In the concentration range of 1.0–8.0 mg/l ($\lambda_{\max} = 251$ nm) the linear dependence equation was $A = 0.1590C$ ($R^2 = 0.9988$), in the range 12.0–70.0 mg/l ($\lambda_{\max} = 285$ nm) $A = 0.0210C$ ($R^2 = 0.9977$), in the range 9.0–55.0 mg/l ($\lambda_{\max} = 309$ nm) $A = 0.0460C$ ($R^2 = 0.9968$).

At the next research stage, spectral curves were differentiated using the UVProbe 2.31 software. Since differentiation reveals finer structure of the original spectrum [14] corresponding changes in the derivatives needed to be established. As the original absorption spectrum of papaverine hydrochloride has two bands of different shape and intensity, the transformation of each band at various $\Delta\lambda$ was studied. The main characteristics for assessing the conformity of derivative shapes to theoretical differentiated curves of orders 1 to 4 were chosen as positions and intensities of extrema, as well as “zero crossings“ (ZC) [12]. Differentiation of the original absorption spectra of solutions with concentrations 6.0 and 12.0 mg/l was performed, varying 16 parameters for each differentiation order: 4 scanning modes of the original spectrum at 4 values of interval $\Delta\lambda$. The influence of registration and differentiation parameters was most clearly visible on resulting curves corresponding to the shortwave asymmetric band. For all derivatives it was found that at $\Delta\lambda = 1$ and 2 nm, the curves lacked a smoothed shape, complicating comparison with the shape of the original absorption curve. At $\Delta\lambda = 80$ nm differentiation was not performed since the original spectrum's wavelength range was 200 nm. Therefore, the comparison of curve shapes was conducted for derivatives obtained at $\Delta\lambda$ from 4 to 40 nm.

First-Order Derivatives

Figure 3 shows first derivatives of the shortwave band of the papaverine hydrochloride absorption spectrum in the 200–300 nm range. It is known that the first derivative of a symmetric Gaussian curve results in a curve having two extrema (positive and negative), corresponding to inflection points on the original curve, with maximum rates of optical density increase and decrease. Zero crossings correspond to the positions of extrema on the original curve [12]. Differentiation of an asymmetric curve, which can be considered the overlap of two Gaussians with different intensities, gives a different pattern [14]. Figure 3 shows that the first derivative of the papaverine hydrochloride spectrum has two extrema of opposite signs and three zero crossing points; the shortwave and longwave parts of the curve differ in shape, consistent with theoretical expectations. Since the shortwave part of the original asymmetric spectrum has a shoulder and the optical density increases non-monotonically, this leads to uneven increase of the derivative $dA/d\lambda$ and arm formation. The longwave part of the original spectrum shows a monotonic decrease in optical density, corresponding to a nearly symmetric peak with a negative extremum. Zero crossing points correspond to two minima and one maximum. However, the obtained curves differ significantly; the derivative shape depends on $\Delta\lambda$.

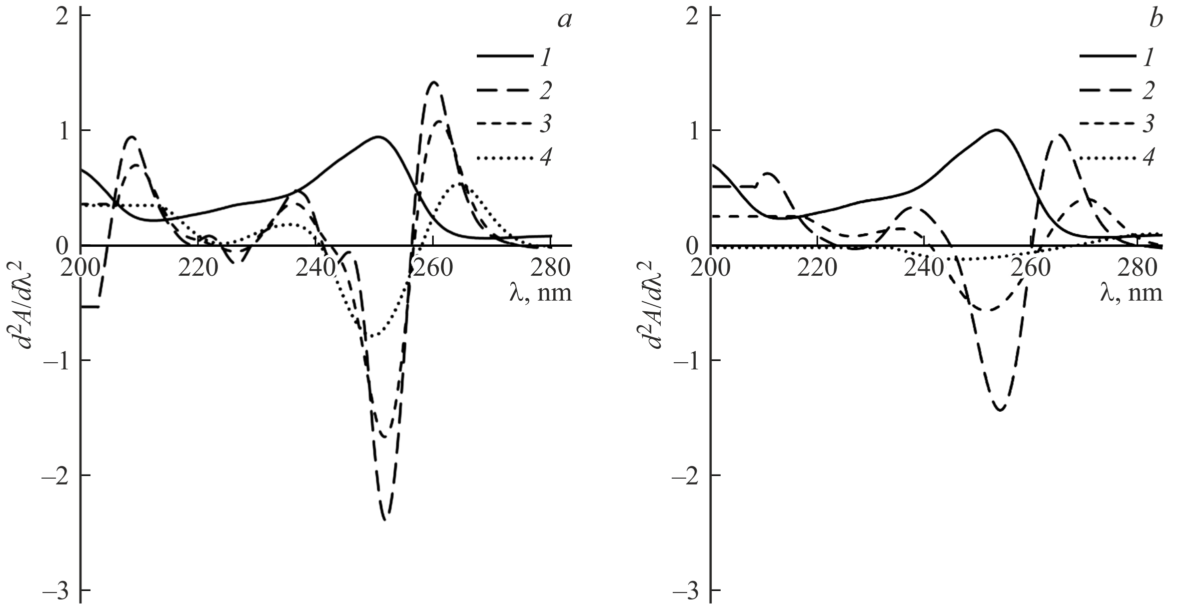

Figure 4. PH absorption spectrum of zero order (*1*) and second derivatives obtained at various $\Delta\lambda$ values: (*a*) $\Delta\lambda = 4$ (*2*), 8 (*3*), 16 nm (*4*) (scanning step 0.2 nm); (*b*) $\Delta\lambda = 10$ (*2*), 20 (*3*), 40 nm (*4*) (scanning step 0.5 nm).

Positions of extrema are closer to the inflection points of the original curve when $\Delta\lambda$ does not exceed 10 nm. A common pattern for all curves is the shift of the positive extremum toward the shortwave region and the negative extremum toward the longwave region. Simultaneously, when $\Delta\lambda$ increases to 20 nm, the derivative values $dA/d\lambda$ decrease almost 2–3 times. The characteristic shoulder of the original spectrum manifests in the derivative either as an arm ($\Delta\lambda = 10$ and 16 nm) or as a distinct low-intensity extremum ($\Delta\lambda = 4$ and 8 nm). At $\Delta\lambda = 20$ and 40 nm the curves practically have no inflections in this range. Of particular interest was the correspondence of zero crossings to the positions at $\lambda_{\max}$ and $\lambda_{\min}$. The closest and coinciding wavelengths of zero crossings with the positions of extrema of the original spectrum were recorded at $\Delta\lambda$ from 1 to 4 nm. As $\Delta\lambda$ increases, the zero crossing position corresponding to $\lambda_{\max}$ shifts to the shortwave region, and that corresponding to $\lambda_{\min}$ shifts to longer wavelengths. This pattern was previously established by us for other compounds [19]. The change in shape of first derivatives with increasing $\Delta\lambda$ has a theoretically obvious explanation, since fewer A values are used to construct the curve over a greater wavelength distance. Likely, the absorption band width, inflection positions, and arm influence this. Small changes in the original spectral curve shape must fall within the differentiation interval.

Second-Order Derivatives

Theoretically, the second derivative of a symmetric curve should have a single narrower intense peak similar to the original curve shape, and two low-intensity satellites. The negative extremum position should correspond to the original spectrum maximum [12,14]. Fig. 4 shows second derivatives of the asymmetric PH absorption spectrum band obtained using the software.

As seen from Fig. 4, increasing the differentiation order leads to systematic amplitude reduction of derivatives. Second derivatives of the absorption spectrum at $\Delta\lambda$ from 4 to 20 nm show some similarity, namely a characteristic intense negative peak and satellites on both sides. Changing the differentiation interval also yields curves with different numerical characteristics of peak and satellite half-widths, amplitudes, and extremum positions. Analysis of the longwave curve part showed that the main peak amplitude exceeds the satellite amplitude by 1.5–2 times. With $\Delta\lambda$ increase, their amplitudes decrease approximately 3.5–4.5 fold and peak broadening occurs. The extremum positions shift to the longwave region: main negative extremum by 3 nm, satellite by 7 nm. The shortwave satellite shape differs from the longwave one; with $\Delta\lambda$ increase it simplifies significantly, amplitude decreases. Notably, changing the differentiation interval also alters the number and positions of ZC. Wavelength values corresponding to ZC positions do not coincide for different differentiation intervals.

Third-Order Derivatives

Third-order differentiation yields a curve with an intense narrow peak and satellites on different sides. For an asymmetric curve, satellite shapes and amplitudes differ. The negative extremum position corresponds to $\lambda_{\max}$ of the original spectrum [12,14]. Fig. 5 shows shapes of obtained third derivatives of the PH absorption spectrum.

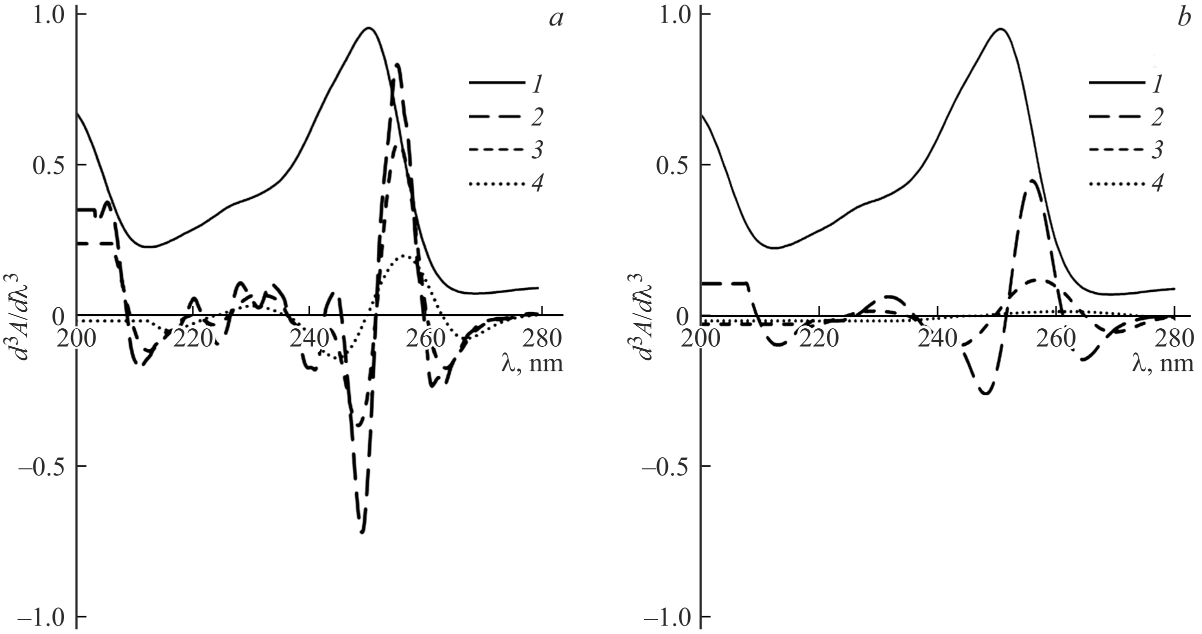

Figure 5. PH absorption spectrum of zero order (*1/*) and third derivatives obtained at various $\Delta\lambda$ values: (*a*) $\Delta\lambda = 4$ (*2*), 8 (*3*), 16 nm (*4*) (scanning step 0.2 nm); (*b*) $\Delta\lambda = 10$ (*2*), 20 (*3*), 40 nm (*4*) (scanning step 0.5 nm).

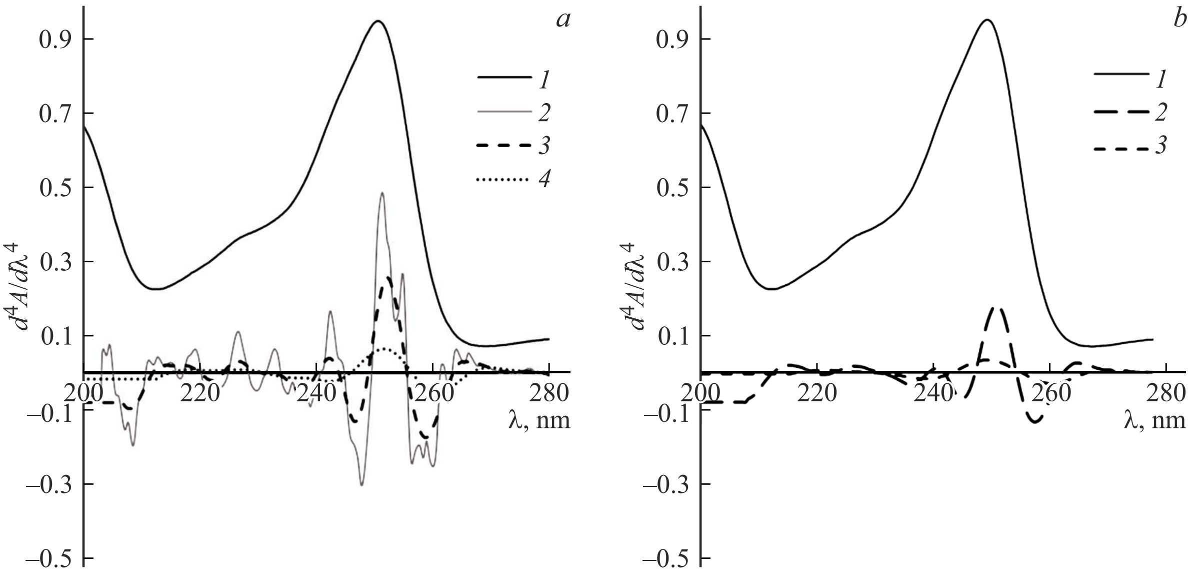

Figure 6. PH absorption spectrum of zero order (*1*) and fourth derivatives obtained at various $\Delta\lambda$ values: (*a*) $\Delta\lambda = 4$ (*2*), 8 (*3*), 16 nm (*4*) (scanning step 0.2 nm); (*b*) $\Delta\lambda = 10$ (*2*), 20 nm (*3*) (scanning step 0.5 nm).

As seen from Fig. 5, curve shapes become significantly more complex, especially in the shortwave region. Third derivative amplitude is lower than first and second derivatives. Values $d^3A/d\lambda^3$ are much lower than absorption intensity in the original spectrum, even with scaling factor. With $\Delta\lambda$ increase, peak amplitudes decrease, curve shapes simplify, ZC and extremum positions change, their number decreases. Closest ZC values corresponding to original spectrum $\lambda_{\max}$ are recorded at $\Delta\lambda$ below 8 nm.

Fourth-Order Derivatives

Fourth-order derivatives have lower amplitude compared to other derivatives. Curve shape becomes more complex, though it should repeat the second derivative shape. The difference lies in reduced half-width of the main peak with simultaneous amplitude reduction of it and surrounding satellites. Fourth-order derivatives are shown in Fig. 6.

As seen from Fig. 6, curve shape complexity already at $\Delta\lambda = 4$ nm omplicates spectral data interpretation. Curves undergo similar transformations with $\Delta\lambda$ increase. Significant amplitude reduction leads to $\Delta\lambda = 40$ nm values near zero at $d^4A/d\lambda^4$ making curve shape practically impossible to display at the given scaling factor.

Thus, scanning spectrophotometer software enables rapid and sufficiently reliable obtainment of first- to fourth-order electronic absorption spectrum derivatives at various differentiation intervals. Curve shapes depend on the set scanning step of the original absorption spectrum and selected $\Delta\lambda$ value.

When applying differentiation via spectrophotometer software for structure determination or compound identification, note that characteristic zero-order spectrum features (extrema, shoulders, inflections, band half-widths) are more clearly revealed in first- and second-order derivatives at low $\Delta\lambda$ values. Increasing differentiation order and reducing $\Delta\lambda$ interval leads to curve shape complexity, potentially complicating decoding. Increasing $\Delta\lambda$ yields simpler curve shapes, risking data misinterpretation.

For quantitative method development, important derivative characteristics include not only shape but also peak amplitudes and ZC positions. Increasing differentiation order and $\Delta\lambda$ reduces $d^nA/d\lambda^n$ potentially lowering analytical method sensitivity.

Varying differentiation parameters allows software to generate derivatives with diverse spectral characteristics, useful for analyzing multicomponent systems with overlapping absorption spectra, enhancing method selectivity.

Method development using absorption spectrum derivatives requires thorough optimization considering scanning and differentiation interval effects. Spectrophotometer instrumental capabilities play a key role in obtaining original optical density values at corresponding wavelengths, as they undergo further mathematical transformations. Differentiation interval selection must be justified based on derivative spectrophotometry application goals.

Obtained results on various instrumental parameter effects on absorption spectrum derivatives may be useful not only for spectrophotometric studies but also other methods employing analytical data differentiation.

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Conflict of interest

The author declares that she has no conflict of interest.

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