

REVIEW

## **Analytical application of derivative spectrophotometry**

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### 1. INTRODUCTION

The derivative method in UV/visible and IR spectrophotometry was introduced in 1953.<sup>1-3</sup> The initial lack of reasonably priced instrumentation and the original limitation to the first derivative are the reason why this technique was accepted only hesitantly. Therefore, derivative spectrophotometry has only recently become a generally applied analytical method, since the rapid progress in the technology of microcomputers has made it possible to directly present the first, the second and higher order derivative spectra.

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The great interest towards derivative spectrophotometry (DS) is due to the increased resolution of spectral bands, allowing the detection and location of the wavelengths of poorly resolved components of complex spectra and reducing the effect of spectral background interferences.<sup>4–6</sup> Because of these characteristics, the processes of isolation and preconcentration of active components, usually required in qualitative and quantitative spectrophotometric procedures applied in the analysis of complex systems, is completely avoided.

The conceptual simplicity, relatively quick and easy realization, increased selectivity and sensitivity in the analysis of minor components are the main reasons why the interest in DS is constantly growing for practical application, as well as for the development of spectrophotometers with integrated computers and accompanying software for producing derivative spectra of different orders. As a result, a great number of papers dealing both with the theoretical aspects of the derivative technique connected with the appropriate numerical analysis and with detailed critical analysis of derivative techniques of certain order have appeared. These papers also describe the application of DS in different fields, *e.g.*, clinical, pharmaceutical, biochemical and environmental analyses, especially of drugs, food and multicomponent organic and inorganic mixtures. General analytical applications of UV/visible derivative spectrophotometry has been reviewed for the period till 1993.<sup>7,8</sup>

As with any measurement technique, it is possible that derivative measurements, if used incorrectly, may actually introduce errors larger than would have been observed without its use. A basic understanding of the derivative concept will minimize this possibility.

The main purpose of this paper is to present the basic characteristics of derivative spectrophotometry and to review the general analytical applications of DS introduced during the last five years (since 1994). In addition, the application of DS for the determination of equilibrium constants is reported.

## 2. BASIC CHARACTERISTICS OF DERIVATIVE SPECTROPHOTOMETRY

### 2.1. Increase of spectra resolution

The main characteristic of DS, the enhancement of the resolution of overlapping spectral bands, is the consequence of differentiation which discriminates against broad bands in favour of a sharp peak to an extent which increases parallel to the derivative order.<sup>5</sup> This property depends on the intrinsic band-width. For two representative simple band shapes, Gaussian and Lorentzian, which are typical of the type encountered in practical spectroscopy, the amplitude in the  $n$ -th derivative order ( ${}^nD$ ) is inversely related to the  $n$ -th power of the band-width ( $W$ ) of the original spectrum:

$${}^nD \propto \frac{1}{W^n} \quad (1)$$

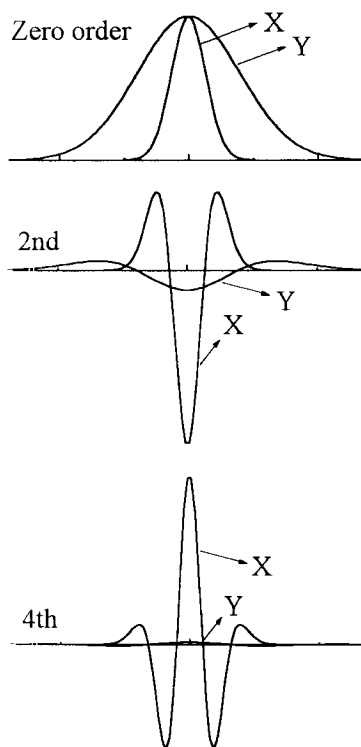


Fig. 1. Effect of derivative order (zeroth, second and fourth) on the relative amplitudes of two coincident Gaussian bands, X and Y, of equal intensity but with a bandwidth ratio 1:3.

Thus, if two bands (X and Y) are of the same intensity, but of different width, the derivative amplitude of the sharper band (X) is greater than that of the broader one (Y) by a factor that increases with increasing derivative order:

$$\frac{{}^n D(X)}{{}^n D(Y)} \propto \left( \frac{W_Y}{W_X} \right)^n \quad (2)$$

The relative increase of the amplitude of the sharper band compared to that of the broader one in higher derivatives, represents the most important factor responsible for the increase of sensitivity and selectivity in DS (Fig. 1). Unfortunately, instrument noise, which increases with derivative order, represents a limiting practical factor.

## 2.2. Elimination of the influence of baseline shift and matrix interferences

Qualitative and quantitative investigations of broad spectra are frequently difficult, especially where the measurement of small absorbances is concerned, because of uncontrollable baseline shift, great blank absorption and matrix interferences, regardless of whether they are caused by irrelevant absorption of light scattering by turbid solutions and suspensions. All these influences can be overcome by derivatisation (Figs. 2 and 3). The order of derivatisation depends on the order of the polynomial function used to describe interferences.<sup>5,9,10</sup> In general, if  $n$

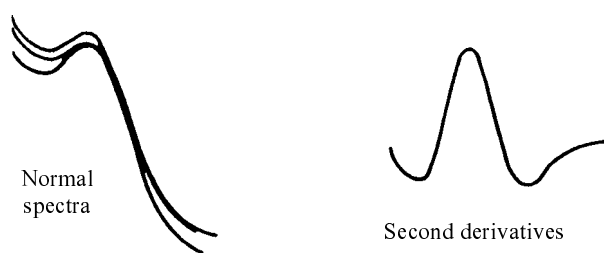


Fig. 2. Reduction of baseline shift and tilt by the derivative technique. *Left*, normal spectra, three scans with different baseline shifts and tilts but identical chromophore absorption: *right*, second derivatives of these three scans superimposed exactly.

represents the highest degree of the polynomial equation used to define an interference, then the interference is reduced to a constant by using the  $n$ -th order derivative and is completely eliminated in the  $(n+1)$ th derivative:

$$P = a_0 + a_1\lambda + a_2\lambda^2 + \dots + a_n\lambda^n \quad (3)$$

$$\frac{d^n P}{d\lambda^n} = n!a_n \quad (4)$$

$$\frac{d^{(n+1)}P}{d\lambda^{(n+1)}} = 0 \quad (5)$$

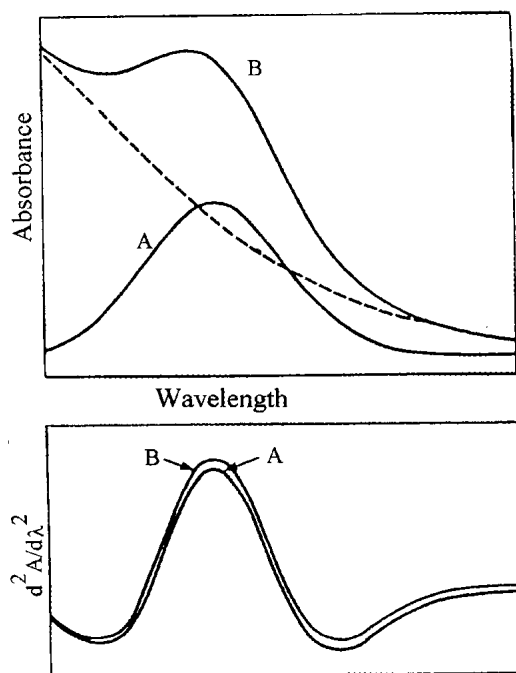


Fig. 3. Reduction in the effect of a curved baseline by the derivative technique. (A) Chromophore absorption alone; (B) observed absorption of chromophore superimposed on baseline; (----) baseline alone.

In many cases, matrix interference can be approximated by a linear or quadratic function. When interference can be described by a linear function ( $P = a\lambda + b$ ), the first derivative yields a function where the interference is reduced to a constant ( $dP/d\lambda = a$ ) and in the second order derivative transformation the interference is completely eliminated ( $d^2P/d\lambda^2 = 0$ ).

### 2.3. Enhancement of the detectability of minor spectral features

Derivatisation of broad spectra increases both the possibility of detection and measurement of minor spectral features and discrimination against interference. Also, it should be kept in mind that derivative transformation of broad spectra does not increase the number of intrinsic data (as a matter of fact, some could be lost as a constant factor) but visually enhances subtle changes in them. Besides qualitative information, this provides wide possibilities for quantitative analysis in cases when the main peak is obscured by an intensive interfering peak (Fig. 3) and for analysis of multicomponent mixtures. Although, a great number of theoretical and practical investigations<sup>11-14</sup> has been developed so far, a general approach to the application of DS in quantitative analysis is impossible, because each combination of bands and the degree of their overlapping tends to be an individual case.

### 2.4. Precise determination of the positions of absorption maxima

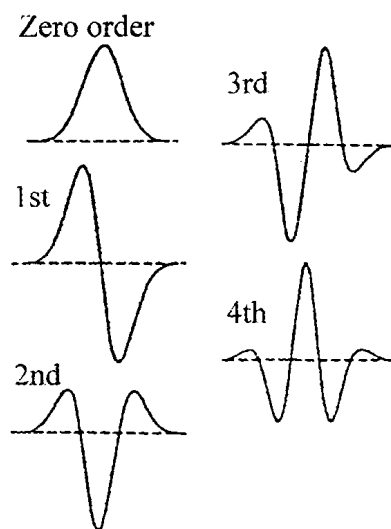


Fig. 4. Characteristic profiles of derivative orders of a Gaussian band.

When a single-peak spectrum has a broad band as its main feature, the position of the absorption maximum can be only approximately determined. The first derivative of this band ( $dA/d\lambda$ ) passes through zero at the peak maximum, minimum and shoulder points (Fig. 4) and can be used to accurately locate the peak position.<sup>15</sup> In contrast, the second and higher even derivatives ( $d^2A/d\lambda^2$ ,  $d^4A/d\lambda^4$ , ...) contain

a peak of changeable sign (negative in the second order, positive in the fourth order, *etc.*) which has the same position as a peak maximum in the normal spectrum. The width of this peak progressively decreases with increasing order of the even derivative, which causes a sharpening of the peak enabling its exact identification. However, every even derivative peak is accompanied by symmetrical satellites of the opposite sign, the number of which is equal to the derivative order. In higher order derivatives ( $n \geq 6$ ) the satellites of adjacent bands may interfere, thus limiting the observed resolution. Also, during differentiation of synthesised spectral profiles, peaks of certain components might be shifted, compared to their original positions.<sup>14</sup>

### 2.5. Signal-to-noise ratio (SNR)

The main disadvantage of the derivative technique is that the *SNR* becomes worse as the order of the derivative increases.<sup>16</sup> A detailed study on the effect of derivatisation on the *SNR*, together with a general approach for the optimisation of the *SNR* on examples of Gaussian and Lorentzian bands has been described by O'Haver.<sup>17,18</sup> The noise of the zeroth-order derivative may be expressed as the standard deviation  $\sigma_0$  of all the elements in a series consisting only of noise, *i.e.*, when there is no signal. The standard deviation of the  $n$ -th order derivative  $\sigma_n$  can be calculated by usual the rules for error propagation. The relative *SNR* of the  $n$ -th derivative without smoothing is given by the following expression:

$$\frac{(SNR)_n}{(SNR)_0} = \frac{C_n}{M^n} \quad (6)$$

where  $C_n$  is a constant which depends on the derivative order and the type of the band;  $(SNR)_0$  represents the *SNR* of the unsmoothed zeroth derivative;  $M$  is the number of points in the peak full-width at half maximum (*FWHM*). For example, if  $M = 10$  the relative *SNR* of the first four derivatives are 0.20, 0.032, 0.008 and 0.0017 in Gaussian bands and 0.18, 0.041, 0.017 and 0.0064 in Lorentzian bands. Clearly, if  $M$  is large, the *SNR* of higher derivatives will be very poor even if the *SNR* of the original spectrum is satisfactory. Therefore, the practical derivative technique includes a certain degree of low-pass filtering or smoothing to control the noise increase which is an inevitable consequence of a noisy signal differentiation. The effect of smoothing a peak-type signal is to reduce the noise, which is desirable and to distort the signal, which is undesirable but unavoidable. The distortion is seen as an attenuation in the peak height and a slight increase in the peak width. The extent of this distortion depends on the smoothing ratio (the ratio of width of the smooth to the *FWHM*) and the number of times that the smoothing is passed through the data series (the sliding average and the quadratic-cubic types of smoothing function require  $n+1$  passes for the  $n$ -th derivative). The trade-off between peak-height attenuation and the relative *SNR* as a function of smoothing ratio is illustrated in Fig. 5 for the second derivative of a Gaussian band.

The selection of the optimum smoothing ratio depends on the purpose for the application of derivative technique. When used for the purpose of resolution

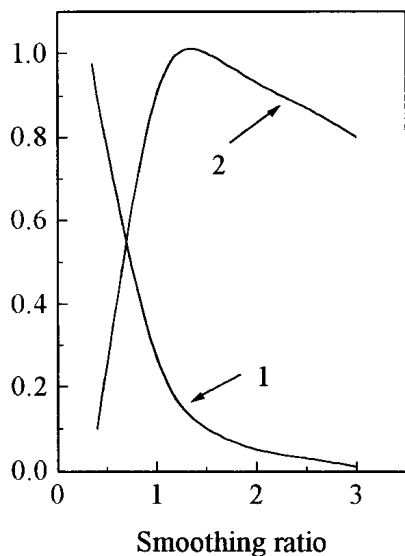


Fig. 5. Attenuation factor (1) and relative signal to noise ratio (2) as a function of the smoothing ratio for the second derivative of a Gaussian band.

enhancement, a relatively small smoothing ratio will assure that a small loss in effective resolution will result. In such a case a significant loss in the *SNR* will have to be tolerated. In quantitative applications, when the derivative technique is used to remove or reduce a broad-band background, significantly larger smoothing ratios may be profitably employed.

Since the origin of noise can be different, depending on both the instrument and the sample, the smoothing process involves optimisation of all available instrumental parameters (full-scale response, slit width, scan speed, absorbance units) to be used for a particular problem on a particular instrument.<sup>10,14</sup>

### 2.6. Quantitative analysis

The application of DS for quantitative analyses is based on the same requirements as normal spectrophotometry, *i.e.*, the validity of Beer's law and the additivity of absorbances.<sup>15</sup> For the derivative spectra of the *n*-th order at a wavelength  $\lambda$ , these laws can be represented by the following equations:

$${}^nD = \frac{d^n A}{d\lambda^n} = \frac{d^n \epsilon}{d\lambda^n} cb \quad (7)$$

$${}^nD(T) = {}^nD(X) + {}^nD(Y) + \dots \quad (8)$$

where *A* is the absorbance,  $\epsilon$  represents the molar absorptivity, *c* the concentration, *b* the path length and  ${}^nD(T)$  the total derivative amplitude, which is equal to the algebraic sum of each absorbing component *X*, *Y*, *etc.* The application of these laws is based on a previous choice of optimal conditions, which include the selection of the most appropriate analytical bands, the suitable derivative order, the method of measurement and optimisation of all significant instrumental parameters. The most

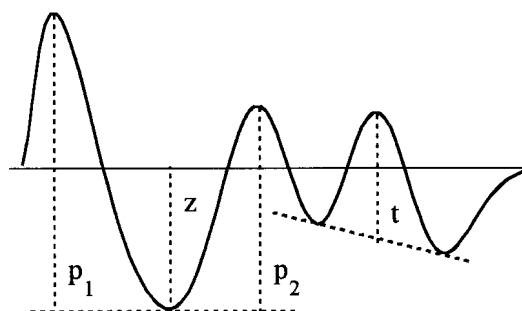


Fig. 6. Graphical measures for amplitudes in derivative spectrophotometry: (p) peak-peak method; (t) peak-tangent method; (z) peak-baseline method.

important methods used for the construction of a calibration curve are: peak-peak, peak-baseline, peak-tangent and zero-crossing (Figs. 6 and 7).<sup>15,19</sup> Sometimes, numerical methods of measurement, such as derivatisation of the "ratio spectra", are used.<sup>20</sup> The measurement method of choice, in practice, would be the one showing the best linear dependence on the concentration of the analyte, a zero or near zero intercept at the origin and be the least influenced by the concentration of any other component.

The usage of more than one derivative order in a particular case, as well as the measurement of the amplitude at several wavelengths, caused the need for the introduction of a notation rule for the amplitude values. Fasandamide and Fell<sup>10</sup> proposed a generally applicable method. The letter *D* is used to indicate that the amplitude of the peak has been measured in the derivative domain. The order of

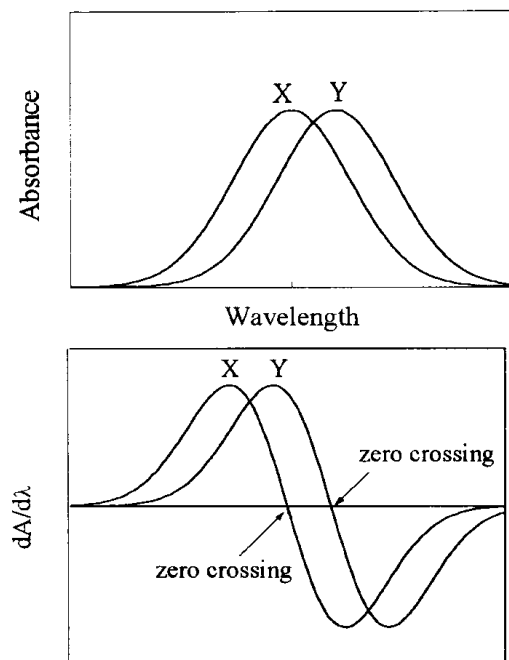


Fig. 7. Use of the zero-crossing technique to allow quantitation of one chromophore (X) overlapped by the absorption band of another chromophore (Y).



derivative is specified by a leading superscript to the letter *D*, e.g.,  $^1D$ ,  $^2D$ ,  $^3D$ . The two wavelengths between which the derivative peak is measured are specified by subscripts separated by a comma. The first wavelength corresponds to the more positive amplitude value while the second one defines position of the more negative value. The peak is measured with respect to the zero baseline at the same wavelength and only one wavelength needs to be specified.

Derivative spectrophotometry is widely applied in inorganic and organic analysis, toxicology, clinical analysis, analysis of pharmaceutical products, amino acids and proteins, in analysis of food and in environmental chemistry. In general, the application of derivative spectrophotometry is not limited to any particular case or field, but can be used whenever qualitative or quantitative investigations of broad spectra are difficult.

### 3. ANALYTICAL APPLICATION

#### 3.1. Inorganic analysis

Due to its increased selectivity and sensitivity compared to classic spectrophotometry, DS is especially widely applied in inorganic chemistry for the simultaneous determination of trace elements of similar chemical properties present in mixtures at different concentration levels. For this purpose, the first and the second order derivative are usually used, although in some cases higher-order derivatives provide more reliable results.<sup>21,22</sup>

Most of the DS methods aimed at the simultaneous determination of inorganic substances were developed till '90s,<sup>7,8</sup> but scientific interest directed towards further developments and improvements of these approaches is still intensive, since DS frequently represents the method of choice for the determination of inorganic substances.

A first-order derivative method was described for the simultaneous determination of mixtures containing nickel, copper and zinc,<sup>23</sup> and cobalt, nickel, copper, zinc and iron<sup>24</sup> using PAR as the chromogenic reagent. The same reagent was applied in a second-order derivative method for the determination of nickel(II) (0.2–1.25 ppm) and cobalt(II) (0.25–1.25 ppm) in a mixture.<sup>25</sup> Mathew *et al.*<sup>26</sup> developed a first-order derivative method for the simultaneous determination of copper (0.0125–0.25  $\mu\text{g/ml}$ ), mercury (0.025–0.25  $\mu\text{g/ml}$ ) and lead (0.025–0.25  $\mu\text{g/ml}$ ) using dithizon as the reagent. Based on the three-component color system of 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol, cetylpyridinium bromide and manganese, copper and zinc in an aluminium alloy, a DS method was developed for the analysis of this alloy.<sup>27</sup> The determination of the aluminium content in some pharmaceuticals was achieved by derivatisation of the spectrum of the complex of aluminium and oxine-5-sulphonic acid at pH 4–5.<sup>28</sup> Also, the estimation of nickel(II) (detection limit 0.2 ng/ml) in the presence of cobalt was performed by applying first-order DS on the complex of nickel and 2-(5-bromo-2-pyridylazo)(5-

diethylamino)phenol in the presence of Triton X-100.<sup>29</sup> In addition, application of DS served to develop methods for Cu(II) (detection limit 4.0 ng/ml) determination in non-ionic micellar medium with 1-(2-pyridylazo)-2-naphthol (PAN) in the presence of the neutral surfactant Triton X-100.<sup>30</sup> The method has been applied in the quality control of numerous commercially available alcohol beverages, biological samples and standard alloys. Taher *et al.*<sup>31</sup> used the same reagent (PAN) to measure iridium after preconcentration of its complex on microcrystalline naphthalene and the application of first order DS diminished the detection limit to 20 ppb. Different parameters significant for the optimization of the experimental conditions have been studied in relation to the determination of iridium in synthetic samples corresponding to various standard alloys and environmental samples. Besides, a very sensitive and selective DS method for the measurement of palladium(II) in the form of its complex with a novel reagent pyridopyridazine dithion (PPD)<sup>32</sup> has been developed.<sup>32</sup> The detection limit determined by normal spectrophotometry was 0.2 µg/ml (0.1 µg/ml in the presence of Triton X-100), while the significantly lower detection limit of 3.7 ng/ml was achieved using the fourth-order derivative mode. The method is free from interference by most common metal ions and anions and it was successfully applied to the determination of palladium present in activated charcoal. A first-order DS method for the simultaneous determination of Pd(II) and Co(II) with diethylenetriamine pentaacetic acid (DTPA) has been developed by Perez-Iglesias *et al.*<sup>33</sup>

Second-order DS was applied in the determination of neodymium, holmium and erbium in mixed rare earths by norfloxacin.<sup>34</sup> The RSDs were 1.0, 1.4 and 1.1% for  $6.9 \times 10^{-5}$  mol/l Nd,  $6.1 \times 10^{-5}$  mol/l Ho and  $6.0 \times 10^{-5}$  mol/l Er, respectively. This procedure was also used to measure small amounts of dysprosium, holmium and erbium.<sup>35</sup> First order DS was applied for the determination of nickel in alloys and biological samples after preconcentration with the ion pair of 2-nitroso-1-naphthol-4-sulphonic acid and tetradecyldimethylbenzylammonium chloride onto microcrystalline naphthalene or by a column method.<sup>36</sup> Detection limit was found to be 0.3 µg/ml. For the optimization of the experimental conditions in the determination of nickel in standard alloys and biological samples, different parameters have been examined. DS methods for the estimation of microamounts of nickel and aluminium in alloys have been developed applying 2-(5-bromo-2-pyridylazo)-5-phenol as the reagent.<sup>37</sup> The matrix Al and co-alloying elements Ti, Mn, Zn, Mg, Pb, Sn, V, Ga, Zr, rare earths, Cu, Fe, Co, were masked with F<sup>-</sup>, tartarate, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, thiourea, pyrophosphate or nitrite. The RDS was <7% and the recovery 97–105%. A derivative double wavelength photometric method was developed for the simultaneous determination of cobalt and nickel in heavy oil.<sup>38</sup> OP-5-Br-PADAP was used as the colour-developing system after the separation of the interfering elements. The RSD for the determination of Co and Ni was 2.8–7.8% and the recovery 94.0–100.0%.

### 3.2. Organic and pharmaceutical analyses

Methods for the determination of organic substances by the DS technique have been developed mainly for application in the analysis of pharmaceuticals and/or

clinically and biochemically interesting systems. The interference of the formulation excipients or other UV-absorbing components, such as co-formulated drugs and degradation products, usual in conventional UV-spectrophotometry can be successfully eliminated by the DS technique.

A variety of procedures that render the DS determination of drugs more specific and sensitive, regardless of whether they are determined as single compounds or in mixtures, have been published.

First- and second-order DS methods have been proposed for the assay of the antiinflammatory drugs fentiazac, flufenamic acid, tiaprofenic acid and proquazone.<sup>39</sup> Similar methods have been developed for the determination of several other drugs alone, such as metronidazole (1–20 µg/ml) in tablets,<sup>40</sup> carboplatin ( $\leq$  150 µg/ml),<sup>41</sup> anthralin in ointments<sup>42</sup> and paracetamol in blood sera.<sup>43</sup>

Aspirin, phenacetin and caffeine in analgesic tablets have been determined by zerocrossing derivative spectrophotometry.<sup>44</sup> Chlorpheniramine maleate, codeine phosphate and ephedrine hydrochloride have been estimated without separation using second-order DS. Chlorpheniramine maleate (0.001–0.08 mg/ml) and codeine phosphate (0.001–0.4 mg/ml) were measured simultaneously, while ephedrine hydrochloride (0.005–1.80 mg/ml) was determined after oxidation with sodium periodate.<sup>45</sup> For quality control of pharmaceutical preparations containing clozapine, two analytical procedures were developed – HPLC (5–150 µg/ml) and DS (5–50 µg/ml) suitable for different levels of the drug.<sup>46</sup>

Numerous papers published so far are related to analyses of drugs in mixtures. Thus, methods for determination of paracetamol and phenoprobamate by first-order DS,<sup>47</sup> mixtures of cocaine, procaine and lidocaine in pulver samples by second-order DS,<sup>48</sup> paracetamol (10–40 µg/ml) and caffeine (1–3 µg/ml) in tablets by first- and second-order DS,<sup>49</sup> acetylsalicylic acid and free salicylic acid in sustained release tablets,<sup>50</sup> cetrimide and chlorhexidine glyconate in antiseptic solutions by first-order DS<sup>51</sup> have been described. Fourth-order DS procedures have been used for the determination of clopramide and pindolol in tablets,<sup>52</sup> lidocaine hydrochloride and 5-nitrox in liquid formulations.<sup>53</sup> also, DS methods have been described for the assay of phenobarbitone in mixtures with oxyphenonium bromide and meprobramate, paracetamol, or acetylsalicylic acid (first and second order),<sup>54</sup> procaine hydrochloride with benzoic acid, pyridoxine hydrochloride, 4-aminobenzoic acid,<sup>55</sup> 0.5–14 µg/ml sulfanilamide and 1–20 µg/ml sulfadiazine (third DS)<sup>56</sup> and sulfamethoxazole and trimethoprim (second order DS).<sup>57</sup>

Derivative procedures reported for vitamin mixtures are concerned with pyridoxine hydrochloride and thiamine hydrochloride in tablets (first and third order),<sup>58</sup> vitamins B<sub>6</sub> (0.2 µg/ml), B<sub>1</sub> (0.46 µg/ml); and B<sub>12</sub> (0.22 µg/ml), uridine 5'-triphosphate (0.2 µg/ml) in injections (second order),<sup>59</sup> and sodium salicylate, thiamine hydrochloride and ascorbic acid in visalicyl tablets (first and second order).<sup>60</sup> First-derivative measurements have been used to determine benzimidazole and cinnamate, as well as benzophenone derivatives in order to characterize sun-

screens in cosmetic formulations.<sup>61</sup> First- and second-order DS have been described for evaluating bilirubin, albumin and oxyhemoglobin in amniotic fluid.<sup>62</sup>

First-order DS was used for the determination of intact ceftazidime (5–50 µg/ml), cefuroxime sodium (5–35 µg/ml) and cefotaxime sodium (5–40 µg/ml) in the presence of their degradation products.<sup>63</sup> Second- and third-order DS were used for the estimation of acyclovir in the presence of guanine (main impurity) and of diloxanide furoate in the presence of diloxanide (a degradation product).<sup>64</sup> The accuracy of the proposed method was found to be better than that of a classical approach.

For a simultaneous determination of acetaminophen and phenobarbital after their extraction from the corresponding suppositories with borate buffer, pH 10, a first-order DS method was developed.<sup>65</sup> In addition, a new spectrophotometric method was elaborated for the simultaneous analysis of a ternary mixture containing metamizole, paracetamol and caffeine.<sup>66</sup> This method is based on the use of the ratio spectrum derivative obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture containing two of the three compounds in the title mixture. This method applied for the assay of tablets was compared with the alternative spectrophotometric method. First- (zero-crossing) and fourth-order (amplitude-baseline) DS methods for determination of triamterene and hydrochlorothiazide, respectively, in combined tablets have also been described.<sup>67</sup> A first-order DS method has been developed for the simultaneous determination of rifamycin SV sodium and lidocaine hydrochloride in injection solutions.<sup>68</sup> The simultaneous determination of ethinyl estradiol and norgestrel in tablets utilizing first-order DS has been reported,<sup>69</sup> as well.

Besides, three accurate and simple methods (first-order DS, simultaneous equation and multicomponent mode) for the simultaneous determination of tinidazole and furazolidone in tablet formulations have been developed.<sup>70</sup>

A method for the simultaneous determination of melatonin-pyridoxine combination in tablets by the zero-crossing technique of the first- and second-order DS has been reported (RSD < 2%). This method was successfully applied for the determination of both drugs present in laboratory prepared mixtures and in tablets.<sup>71</sup> For the evaluation of diclofenac and benzyl alcohol as an excipient in injectable formulations, the first- and second-order DS method using the zero-crossing technique has been described.<sup>72</sup> In addition, three new methods (first-order DS, ratio spectra DS and Vierordt's method) for the quantitative analysis of tablet formulations containing pseudoephedrine hydrochloride and triprolidine hydrochloride were developed and compared.<sup>73</sup> A rapid, simple and direct assay procedure based on first-order DS using zero-crossing and peak-to-base measurements for the determination of dextromethorphan HBr (detection limit 0.033 µg/ml) and bromhexine HCl (detection limit 0.103 µg/ml) has also been developed.<sup>74</sup> Further, a simple and economical DS procedure was developed for the simultaneous determination of indomethacin and paracetamol in combined dosage forms.<sup>75</sup> Applying the zero-crossing technique of the second-order DS a method for the determination of 1,4-benzodiazepin, midazolam<sup>76</sup> and lorazepam<sup>77</sup> in tablets was developed. Midazolam was estimated in the presence of maleic acid as a coformulation, while lorazepam was measured in the presence of degradation products.

Finally, the simultaneous derivative spectrophotometric technique was applied for the determination of various organic compounds, such as the pesticides atrazine (1–15  $\mu\text{g/ml}$ ), diuron (1–10  $\mu\text{g/ml}$ ) and chlorpyrifos (1–10  $\mu\text{g/ml}$ ) in groundwaters and soil,<sup>78,79</sup> sodium *o*-nitrophenolate, sodium *p*-nitrophenolate and 2-methoxy-5-nitrophenolate in plant and animal growth regulators,<sup>80</sup> tyrosine, tryptophan and phenylalanine<sup>81</sup> and phenol and cresol in the presence of pyrocatechol and resorcinol.<sup>82</sup> Using the first- and second-order derivatives of the spectra ratio, a method for the analysis of binary mixtures of the flavonoids chrisin and quercetin has been described.<sup>83</sup> A third-derivative method using the zero-crossing technique has been employed for the simultaneous determination of (dimethylamino)-ethyl[*o*-chloro-*p*-(dimethylamino)sulfonylphenoxy]acetate hydrochloride and its major hydrolytic decomposition product *o*-chloro-*p*-(dimethylamino)sulfonylphenoxy acetic acid.<sup>84</sup> A multiwavelength linear regression derivative spectrophotometric method for the determination of phenol, hydroquinone and catechol has also been described.<sup>85</sup>

### 3.3. Analysis of food and water

Various derivative methods have been developed for the analysis of food. A simple extraction-first order DS method for the determination of tartrazine (up to 3.0  $\mu\text{g/ml}$ ) and Sunset Yellow (up 3.6  $\mu\text{g/ml}$ ) in commercially available products has been described.<sup>86</sup> First-order DS was applied also for the determination of tartrazine in the presence of amaranth or carmoisine in different sugar candy samples.<sup>87</sup> A very simple method for resolving ternary mixtures of food colorants, such as tartrazine, Sunset Yellow and Ponceau 4R, by using the first derivative of the ratio spectra with measurements at the zero-crossing wavelength has been described.<sup>88</sup> This method was applied for the analysis of synthetic mixtures of these colorants in different ratios with recoveries in the 94–105% range. Several other derivative methods have dealt with the simultaneous determination of colorants and dyes in mixtures such as carminic acid, riboflavin and erythrosine in yoghurt samples<sup>89</sup> and tetrazine, riboflavin, curcumin and eritrosine.<sup>90</sup>

For detection of aromatic hydrocarbons in water samples, derivatives of the transmission spectrum with respect to wavelength were used.<sup>91</sup> This method has an enhanced signal-to-noise ratio due to the generation of the derivatives in an optical manner. As examples of the application, monitoring of water samples for the presence of aromatic hydrocarbons, *e.g.*, benzene, toluene and xylene, as well as process control in the chemical industry, were described.

### 3.4. Application of derivative spectrophotometry for the determination of equilibrium constants

It is rather difficult to apply classical spectrophotometric methods for the determination of ionization constants when the ionization of the examined compounds is accompanied by minor changes in the absorption spectra. To solve this problem, the application of DS can be very suitable because the increased resolution of DS enables both the detection and determination of components in multicomponent systems even if their spectra overlap strongly.

Levillain and Fompeydie<sup>92</sup> used this DS characteristic to study the acid-base equilibrium of eosin which represents a diprotic acid with strong overlapping of both the spectra and the ionization constants. Due to the fact that the molecular form of eosin (EH<sub>2</sub>) is colorless, these authors applied first-order DS employing zero-crossing measurements to monitor the pH-dependent concentration of the mono- (EH<sup>-</sup>) and dianionic (E<sup>2-</sup>) forms of eosin and the values obtained served to calculate the equilibrium constants.

In contrast to the method proposed which is limited to zero-crossing measurements, the most general DS method for the determination of acidity constants, free of limitations with respect to the order of the derivative and the method of acidity constants, free of limitations with respect to the order of the derivative and the method of measurement has been developed by Popović and Pfendt.<sup>93</sup> This method is based on the same conditions as classical spectrophotometry, *i. e.*, on the validity of Beer's law and absorbance additivity. Various variations of the proposed method, which make the determination of the constants possible by using different measuring methods (peak-base line and zero-crossing methods), are given.

#### SUMMARY

The general aspects of derivative spectrophotometry (DS) and its advantages and limitations with respect to normal spectrophotometry are considered. A review of the analytical application of DS in different fields, *e. g.*, inorganic, organic, pharmaceutical, environmental and food analysis as well as chemical equilibria, is given.

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#### ИЗВОД

#### АНАЛИТИЧКА ПРИМЕНА ДЕРИВАТИВНЕ СПЕКТРОФОТОМЕТРИЈЕ

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Разматрани су основни принципи деривативне спектрофотометрије (DS) и њене предности и ограничења у односу на нормалну спектрофотометрију. Дат је преглед аналитичке примене DS у различитим областима: неорганској и органској анализи, анализи фармацеутских препарата, клиничкој анализи, анализи хране, хемије животне средине, хемијској равнотежи.

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#### REFERENCES

1. V. J. Hammond, W. C. Price, *J. Opt. Soc. Am.* **43** (1953) 924
2. E. Tannebauer, P. B. Merkel, W. H. Hamill, *J. Phys. Chem.* **21** (1953) 311
3. J. D. Morrison, *J. Chem. Phys.* **21** (1953) 1767
4. G. Talsky, L. Mayring, H. Kreuzer, *Angew. Chem.* **17** (1978) 785
5. T. C. O'Haver, *Anal. Chem.* **51** (1979) 91A
6. A. F. Fell, G. Smith, *Anal. Proc.* **19** (1982) 28

7. F. S. Rojas, C. B. Ojeda, J. M. C. Pavon, *Talanta* **35** (1988) 753
8. C. B. Ojeda, F. S. Rojas, J. M. C. Pavon, *Talanta* **42** (1995) 1195
9. T. C. O'Haver, *Clin. Chem.* **25** (1979) 1548
10. A. A. Fasanmade, A. F. Fell, *Analyst* **110** (1985) 1117
11. J. R. Morrey, *Anal. Chem.* **40** (1968) 905
12. B. F. Barker, M. F. Foz, *Chem. Soc. Rev.* **9** (1980) 143
13. T. C. O'Haver, G. L. Green, *Anal. Chem.* **48** (1976) 312
14. T. R. Griffiths, K. King, H. V. St.A. Hubbard, M. J. Schwing-Weill, J. Meullemeestre, *Anal. Chim. Acta* **143** (1982) 163
15. J. M. Battenbury, *Amino Acid Analysis*, Halesed-Wiley Press, Chichester, 1981, p. 92
16. J. E. Cahill, *Am. Lab.* **11** (1979) 79
17. T. C. O'Haver, *Anal. Proc.* **19** (1982) 22
18. T. C. O'Haver, T. Begley, *Anal. Chem.* **53** (1981) 1876
19. B. Morelli, *Analyst* **113** (1988) 1077
20. F. Salinas, J. J. B. Nevado, A. E. Mansilla, *Talanta* **37** (1990) 347
21. S. Kus, Z. Marczenko, *Analyst* **112** (1987) 1503
22. G. Talsky, S. Götz-Maler, H. Betz, *Mikrochim. Acta (Wien)* **II** (1981) 1
23. L. Baoshan, *Shiyou Huagong Gaodeng Xuexiao Xuebao* **7** (1994) 25 [*C.A.* **123** (1995) 73644f]
24. L. Zhang, L. Li, B. Li, L. Wang, Q. Lu, H. Zhang, M. Lium, Q. Jin, *Jilin Daxue Ziran Kexue Xuebao* **1** (1995) 104 [*C.A.* **123** (1995) 73614w]
25. W. Ho, C. Chen, H. Chen, *J. Chin. Chem. Soc. (Taipei)* **42** (1995) 51
26. L. Mathew, T. P. Rao, C. S. P. Iyer, A. D. Damodaran, *Mikrochim. Acta* **118** (1995) 249
27. B. Ma, L. Liu, D. Liu, S. Chen, *Lihua Jianyan, Huaxue Fence* **30** (1994) 337 [*C.A.* **123** (1995) 131509d]
28. Z. Zhag, W. Liang, *Shandon Daxue Xuebao, Ziran Kexuebao* **31** (1996) 202 [*C.A.* **125** (1995) 291759e]
29. S. L. C. Ferreira, A. C. Spinola Costa, D. S. de Jesus, *Talanta* **43** (1996) 1649
30. N. K. Agnihotri, V. K. Singh, H. B. Singh, *Talanta* **45** (1997) 331
31. M. Taher, S. Puri, R. K. Bansal, B. K. Puri, *Talanta* **45** (1997) 411
32. A. Y. El-Sayed, F. A. Abu-Shanab, *Mikrochim. Acta* **129** (1998) 225
33. J. Perez-Iglesias, H. Seco-Lago, J. Fernandez-Solis, J. Castro-Romero, V. Gonzalez-Rodriguez, *Ann. Chim (Rome)* **88** (1998) 597
34. N. X. Wang, L. Wang, W. Jiang, Y. Z. Ren, Z. K. Si, X. X. Qui, G. Y. Du, P. Qi, *Fresenius 'J. Anal. Chem.* **361** (1998) 821
35. M. Anbu, T. P. Rao, C. S. P. Iyer, A. D. Damodaran, *Chem. Anal. (Warsaw)* **41** (1996) 781
36. M. Ali Taher, A. Mostafavi Dehzoeei, B. Krishan Puri, S. Puri, *Anal. Chim. Acta* **367** (1998) 55
37. P. Lu, *Lihua Jianyan, Huaxue Fence* **34** (1998) 22
38. J. Ma, L. Liu, P. Li, G. Liu, *Lihua Jianyan, Huaxue Fence* **34** (1998) 164 [*C.A.* **129** (1998) 339165 v]
39. M. H. Barary, S. M. Blaih, H. H. Abdine, M. A. El Sayed, *Maansoura J. Pharm. Sci.* **10** (1994) 1 [*C.A.* **121** (1994) 213141a]
40. H. Zhang, Q. Chen, W. Wang, Z. Shao, *Z. Zhongguo Yiyao Gongye Zashi* **26** (1995) 72 [*C.A.* **122** (1995) 322650g]
41. P. Wei, G. Mai, H. Sui, *Fenxi Huaxue* **23** (1995) 486 [*C.A.* **123** (1995) 93441r]
42. M. T. Orbey, S. Aydogan, N. Gunden Goger, *J. Fac. Pahrn. Gazi Univ.* **11** (1994) 127
43. P. C. Damiani, M. E. Ribone, A. C. Olivieri, *Anal. Lett.* **28** (1995) 2219
44. X. Li, X. Bai, X. Peng, J. Niu, D. Zhao, *Zhongguo Yaoxue Zazhi* **30** (1995) 743 [*C.A.* **124** (1996) 242468e]
45. F. Sayin, S. Kir, A. Temizer, *FABAD Farm. Bilimler Derg.* **21** (1996) 55
46. M. A. Raggi, F. Bugamelli, G. Casamenti, G. Giovanninetti, V. Volterra, *Pharmazie* **53** (1998) 452
47. C. Yucesoy, K. Tulek, *Egypt. J. Anal. Chem.* **3** (1994) 156 [*C.A.* **122** (1995) 223012u]
48. A. Cruz, M. Lopez Rivadulia, A. M. Bermejo, I. Sanchez, P. Fernandez, *Anal. Lett.* **27** (1994) 2663

49. M. Surmeian, D. Georgescu, V. V. Cosofret, *Rev. Chim. (Bucharest)* **45** (1994) 823
50. G. Jovičić, L. Nikolić, K. Karljiković Rajić, D. Agbaba, M. Jovanović, Z. Djurić, *Farmaco* **50** (1995) 285
51. E. Dinc, F. Onur, *J. Fac. Pharm. Gazi Univ.* **11** (1994) 135
52. I. Panderi, M. Parissi Poulou, *Int. J. Pharm.* **99** (1993) 327
53. A. V. Pavlova, G. I. Ljutakov, A. N. Ziatinova, *Anal. Lab.* **4** (1995) 42
54. H. Salem, A. Aboul Kheir, *Zagazig J. Pharm. Sci.* **4** (1995) 117 [*C.A.* **123** (1995) 266287a]
55. M. Surmeian, G. Ciohodaru, M. Ionescu, V. Cosofret, *Rev. Roum. Chim.* **40** (1995) 111
56. J. Berzas Hevado, F. Salinas, I. de Orbe, L. F. Capitan Vallvey, *Afinidad* **52** (1995) 119
57. C. Altesor, P. Corbi, I. Dol, M. Knochen, *Analyst* **188** (1993) 1549
58. I. I. Hewala, *Anal. Lett.* **26** (1993) 1549
59. B. Morelli, *J. Pharm. Sci.* **84** (1995) 34
60. H. Salem, M. El Maamli, M. El Sadek, A. Aboul Kheir, *Zagazig J. Pharm. Sci.* **4** (1995) 175 [*C.A.* **123** (1995) 266291x]
61. P. Michaud, P. Soto, T. Le Roy, F. Rodriguez, *Int. J. Cosmet. Sci.* **16** (1994) 93
62. C. Vetsschi, G. Ragni, *Anal. Lett.* **28** (1995) 605
63. K. Kelani, L. I. Bebawy, L. Abdel-Fattah, *J. AOAC Int.* **81** (1998) 386
64. H. G. Daabees, *Anal. Lett.* **31** (1998) 1509
65. G. Alpdogan, S. Sungur, *Sci. Pharm.* **65** (1997) 247
66. E. Dinc, F. Onur, *Anal. Chim. Acta* **359** (1998) 93
67. V. Ulvi, *Pharm. Pharmacol. Commun.* **4** (1998) 193
68. F. Onur, S. Gungor, *Sci. Pharm.* **65** (1997) 155
69. S. Tatar, S. Atmaa, *Pharmazie* **51** (1996) 251
70. S. Ravisankar, M. J. Nanjan, M. Vasudevan, B. Duraiswamy, B. Suresh, N. Noorudeen, *Indian J. Pharm. Sci.* **60** (1998) 116
71. H. H. Abdine, A. A. Gazy, M. H. Abdel-Hay, *J. Pharm. Biomed. Anal.* **17** (1998) 379
72. Y. C. Micalizzi, N. B. Pappano, N. B. Debattista, *Talanta* **47** (1998) 525
73. E. Dinc, F. Onur, *S. T. P. Pharma Sci.* **8** (1998) 203
74. V. Tantishaiyakul, C. Poeaknapo, P. Sribun, K. Sirisuppanon, *J. Pharm. Biomed. Anal.* **17** (1998) 237
75. S. Gangwal, P. Trivedi, *Indian Drugs* **35** (1998) 291
76. L. B. Pfendt, G. V. Popović, V. M. Stefanović, M. B. Rajić, *J. Serb. Chem. Soc.* **62** (1997) 687
77. L. B. Pfendt, V. M. Stefanović, G. V. Popović, *Arh. farm.* **1** (1999) 27
78. M. M. Galera, J. L. M. Vidal, A. G. Frenich, P. Parrilla, *Analyst* **119** (1994) 1189
79. M. M. Galera, J. L. M. Vidal, A. G. Frenich, P. P. Vazquez, *J. AOAC Int.* **78** (1995) 423
80. J. Li, S. Ma, Y. Wen, *Fenxi Ceshi Huebao* **12** (1993) 55 [*C.A.* **120** (1994) 263667b]
81. L. Micio, E. Perrin, A. Driou, M. Mellet, *Int. J. Pept. Protein Res.* **46** (1995) 186
82. I. Baranowska, A. Skotniczna, C. Pieszko, *Pol. J. Appl. Chem.* **39** (1995) 137
83. Z. Nikolovska-Coleska, Lj. Klisarova, Lj. Suturkova, K. Dorevski, *Anal. Lab.* **5** (1996) 98
84. M. Surmeian, G. Ciohodaru, St. Cilianu, *Anal. Lett.* **29** (1996) 2153
85. C. He, Z. Wang, L. Chen, *Fenxi Shiyanshi* **15** (1996) 25 [*C.A.* **125** (1996) 315832b]
86. P. L. Lopez-de-Alba, L. I. Michelini-Rodriguez, K. Wrobel, J. Amador-Hernandez, *Analyst* **122** (1997) 1575
87. Y. Ozdemir, S. Sayar, A. A. Akkan, *Shim. Acta Turc.* **26** (1998) 35
88. B. J. J. Nevado, R. J. Flores, G. C. Cabanillas, V. M. J. Llerena, C. A. Salcedo, *Talanta* **46** (1998) 933
89. B. J. J. Nevado, G. C. Cabanillas, C. A. M. Salcedo, *Talanta* **41** (1994) 789
90. B. J. J. Nevado, R. J. Flores, V. M. J. Llerena, *J. Fresenius' J. Anal. Chem.* **350** (1994) 610
91. F. Vort, M. Tacke, E. Wagner, *Tech. Mess.* **65** (1998) 293
92. P. Levillain, D. Fompeydie, *Anal. Chem.* **57** (1985) 2561
93. G. V. Popović, L. B. Pfendt, V. M. Stefanović, *Yugoslav Congress of Food, Pharmaceuticals and Chemical Engineering*, Novi Sad (1999) Proceedings, 227.