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An application of second-order UV-derivative spectrophotometry for study of solvolysis of a novel fluocinolone acetonide ester

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ABSTRACT

A novel topical corticosteroid FA-21-PhP, 2-phenoxypropionate ester of fluocinolone acetonide, has been synthesized in order to investigate the possibility of decreasing systemic side effects. In this study model system for in vitro solvolytic reaction of FA-21-PhP has been analyzed in ethanol/water (90:10, v/v) with excess of sodium hydrogen carbonate. The selected conditions have been used as in vitro model for activation of corticosteroid C-21 ester prodrug. The second-order derivative spectrophotometric method (DS) using zero-crossing technique was developed for monitoring ternary mixture of solvolysis. Fluocinolone acetonide (FA) as a solvolyte was determined in the mixture in the concentration range 0.062–0.312 mM using amplitude $^2D_{274.96}$. Experimentally determined LOD value was 0.0295 mM. The accuracy of proposed DS method was confirmed with HPLC referent method. Peak area of parent ester FA-21-PhP was used for solvolysis monitoring to ensure the initial stage of changes. Linear relationship in HPLC assay for parent ester was obtained in the concentration range 0.054–0.54 mM, with experimentally determined LOD value of 0.0041 mM. Investigated solvolytic reaction in the presence of excess of $NaHCO_3$ proceeded via a pseudo-first-order kinetic with significant correlation coefficients 0.9891 and 0.9997 for DS and HPLC, respectively. The values of solvolysis rate constant calculated according to DS and HPLC methods are in good accordance 0.038 and 0.043 h^{-1}, respectively.

1. Introduction

Fluocinolone acetonide (FA) is a corticosteroid used topically for its glucocorticoid activity in the treatment of various skin disorders. Fluocinolone acetonide has a medium and high potency ranking, based on the drug concentration, type of vehicle used and the vasoconstrictor assay as a measure of topical antiinflammatory activity [1].

Pörtner et al. [2] investigated the relative receptor affinities of glucocorticoid-21-esters and found that those esters are true prodrugs for which the glucocorticoid actions are caused only by corresponding glucocorticoid alcohols.

Studies have shown that C-17 or/and C-21 esterified corticosteroids undergo hydrolysis in aqueous and biological media, which is one of the common degradation pathways of steroids and will have an impact on its local potency and the systematic availability. For steroid esters used for a very long time in therapy, it has been established that betamethasone 17-valerate [3] and hydrocortisone butyrate [4] showed pH dependent hydrolysis and/or reversible acyl migration between C-21- and C-17-hydoxy groups in aqueous media. Recently, among several stability-indicating HPLC methods which have been reported in the literature, two papers are of interest illustrating degradation kinetics of mometasone furoate [5] and dexamethasone related substances [6].

Applied topically, particularly to large areas, when the skin is injured, or under occlusive dressings, corticosteroids may be absorbed in sufficient amounts to cause systemic effects [7]. It is known that fluocinonide (FA-21-Ac) is four times more potent than its active form due to enhanced lipophilicity and percutaneous absorption [8].

In order to increase benefit/risk ratio [8], a new steroid compound fluocinolone acetonide 21-(2-phenoxypropionate) (FA-21-PhP) has been synthesized in our laboratory. The chemical name of the novel ester (Fig. 1) is (6α,11β,16α)-6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-pregna-1,4-diene-3,20-dione 21-(2-phenoxypropionate).

The extent of corticosteroid-21-esters hydrolysis depends on the structure of acid radicals [2,9]. Esterases in vivo produce corresponding alcohols and acids. Solvolysis using $NaHCO_3$ or phosphate buffer in alcohol/water medium generates in vitro corresponding esters of acids.

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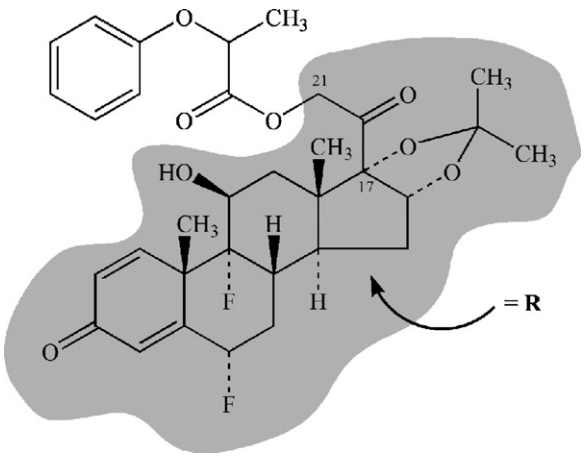


Fig. 1. Structure of fluocinolone acetonide 21-(2-phenoxypropionate) (FA-21-PhP).

One of the classic analytical problems is the simultaneous determination of two or more compounds in the same sample without previous separation. Derivative spectrophotometry (DS) in the UV–vis region is an efficient technique for simultaneous determination by improving spectral resolution of overlapped signals and elimination of background caused by presence of other components or sample matrix. DS increases the detectability of minor spectral features due to discrimination against broad bands in favour to sharp peaks allowing the enhancement of selectivity and sensitivity in multicomponent analysis.

Since 1995, several reviews have been published [10–14], comprising theoretical and instrumental aspects as well as analytical applications in pharmaceutical, biomedical environmental, food and cosmetics analyses. Besides mixture analysis DS has been applied for determination of reaction equilibria with calculation of binding or complexation constants, as well as for investigations of different reaction kinetics. Recently, stability-indicating methods including derivative spectrophotometry for determination of clopidogrel bisulfate [15], as well as photostability of levomepromazine hydrochloride [16] have been reported.

The goal of this paper was to investigate the application of derivative spectrophotometry for the analysis of FA-21-PhP solvolysis in ethanol/water solution using sodium hydrogen carbonate. HPLC method was used as a referent method.

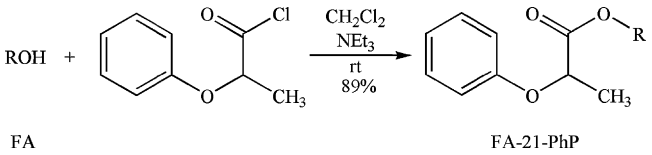
2. Experimental

2.1. Reagents

Fluocinolone acetonide 21-(2-phenoxypropionate) FA-21-PhP was synthesized in our laboratory by reaction of FA and 2-phenoxypropanoyl chloride.

2-Phenoxypropanoyl chloride was prepared in following manner: 2-phenoxypropanoic acid (PhPA) (3.00 g, 18.07 mmol) was dissolved in dichlormethane (3 ml) and the mixture was stirred under nitrogen. A drop of dimethylformamide was added, followed by thionyl chloride (3.23 g, 27.11 mmol) which was added dropwise over 3 min and then the reaction solution was stirred at room temperature overnight. The reaction solution was concentrated in vacuo and the resulting pale yellow oil was placed under high vacuum to remove traces of thionyl chloride to afford pure 2-phenoxypropanoyl chloride (800 mg, 24%).

FA (220.0 mg, 0.486 mmol) was dissolved in dichlormethane (15 ml) and then cooled at 0 °C under nitrogen atmosphere. Triethylamine (0.1 ml, 0.721 mmol) was added in one portion, followed by dropwise addition of 2-phenoxypropanoyl chloride (134.6 mg, 0.729 mmol) over 5 min. The ice bath was removed and the resulting mixture was stirred 4 h at room temperature (Scheme 1). Dichlormethane (50 ml) was added and the solution washed with hydrochloric acid (1 M, 50 ml), aqueous solution of sodium hydrogen carbonate (5%, 50 ml) and water (twice with 50 ml) and dried with Na_2SO_4. The crude product was purified by silica gel chromatography using chloroform as mobile phase to afford the white crystals of equimolar mixture of FA-21-PhP diastereomers (259.8 mg, 89%). The purity (>98%) of synthesized FA-21-PhP was confirmed by IR, HPLC-MS, ^{1}H NMR, ^{13}C NMR and elemental analysis.



Scheme 1. Synthesis of fluocinolone acetonide 21-(2-phenoxypropionate).

Ethyl 2-phenoxypropionate (EtPhP) was synthesized according to a typical procedure from PhPA (2.5 g, 15.04 mmol) and abs. ethanol (10 ml) in excess with a drop of conc. H_2SO_4, during 2 h at reflux. The excess of ethanol was removed under the reduced pressure at room temperature. The residue was partitioned between solvent ethyl acetate:saturated $NaHCO_3$ aqueous solution (50:50, v/v) and separated. Organic layer was washed twice with water and ethyl acetate was removed under the reduced pressure to afford the pure EtPhP (1.5 g, 51%). The purity (>98%) of synthesized EtPhP was confirmed by HPLC and GC-MS.

Reagents fluocinolone acetonide ≥98%, absolute ethanol Chromasolv HPLC purity, methanol Chromasolv HPLC purity (Sigma–Aldrich Chemie GmbH, Steinheim, Germany); 2-phenoxypropionic acid (Fluka Chemie GmbH, Bruch, Switzerland); thionyl chloride for synthesis, chloroform for analysis, sodium hydrogen carbonate, anhydrous sodium sulfate for analysis, silica gel 60 for column chromatography (Merck, Darmstadt, Germany); dichlormethane for analysis, ethyl acetate for analysis (Carlo-Erba, Rodano, Italy); N,N-dimethylformamide (Alfa-Aesar GmbH, Karlsruhe, Germany); triethylamine pure (Lach-Ner, Neratovice, Czech Republick) and deionized water (TKA water purification system, Niederelbert, Germany) were used through out this study.

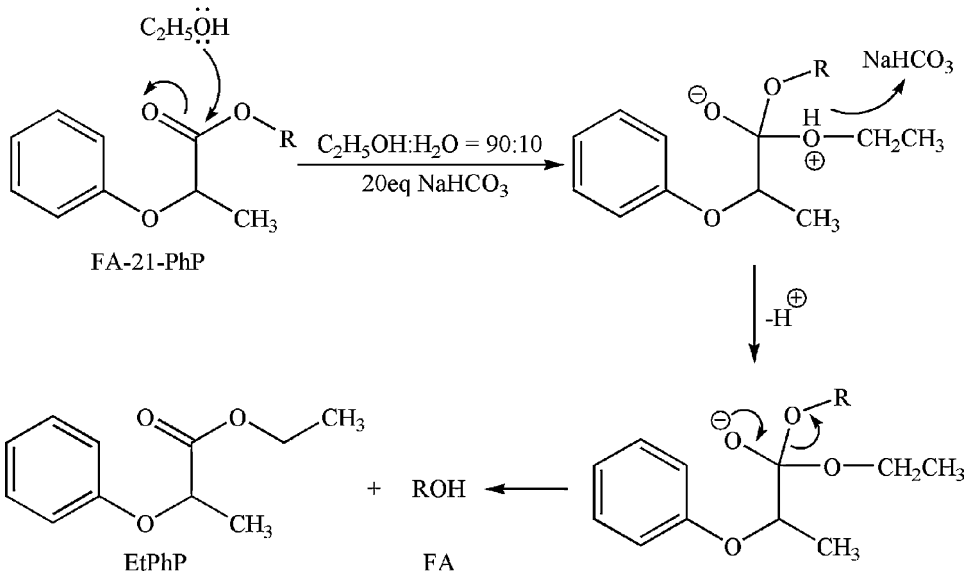
2.2. Instrumentations

2.2.1. Proposed DS method

Absorption spectra were recorded over the wavelength 250–400 nm in 1 cm quartz cells using a GBC UV/visible spectrophotometer Cintra 20 (GBC Scientific Equipment Pty Ltd., Dandenong, Australia). Data processing Spectral Software Ver. 1.70 was used to calculate digital derivative spectra (the first- and second-order spectra were generated directly, but the fourth-order spectra were calculated as the second derivative of second-order spectra). The zero-order spectra were recorded at a scan speed 100 nm min^{-1} and the fixed value of slit width of 1 nm. For calculation of derivative spectra the data interval of 0.48 nm was selected. The second-order derivative spectra were calculated using Savitzky-Golay algorithm with quadratic function for data smoothing with selected 17 points.

2.2.2. Referent HPLC method

HPLC analysis was performed on a Hewlett-Packard (Beaverton, OR) system equipped with binary pump, Rheodyne injector (sample loop 20 μl), 1100A UV detector, controlled by IBM PC Pentium Vectra XA computer.



Scheme 2. The pathway of FA-21-PhP solvolysis.

Analyses were made on a Zorbax Extend-C_{18} column (150 mm × 4.6 mm, i.d., 3.5 μm particle size) Agilent, USA, using isocratic elution with methanol:water (75:25, v/v) at a flow rate of 1 ml min^{-1}. The experiment was conducted at 25 °C. The data were analyzed using the Chem Station software package.

Solvolysis sample mixture and blank solution were stirred with Magnetic stirrer Tehtnica Rotamix SHP-10 (Zelezniki, Slovenia). Radiometer model PHM 240 pH/ION-meter was used.

2.3. Stock solution

Stock solution of FA-21-PhP was prepared by dissolving 20 mg of compound in ethanol in 10 ml volumetric flask. This solution was stable for seven days when stored at room temperature.

Stock solution of FA was prepared by dissolving 15 mg of compound in ethanol in 10 ml volumetric flask. This solution was stable for seven days when stored at room temperature.

Stock aqueous solution of sodium hydrogen carbonate (0.067 M) was prepared by dissolving 280 mg of sodium hydrogen carbonate in 50 ml volumetric flask.

2.4. Procedures for calibration curves

DS-calibration solutions were prepared by transferring 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 ml aliquots of FA stock solution into 10 ml volumetric flask followed with 5 ml of ethanol, then 1.0 ml of sodium hydrogen carbonate stock aqueous solution was added and filled up to mark with ethanol. Zero-order spectra of each calibration solutions have been recorded immediately after preparation under the conditions cited in Section 2.2 (Section 2.2.1). Amplitude $^2D_{274.96}$ of FA was used for regression analysis. Limit of detection for FA was experimentally determined using the concentration 0.0312 mM.

HPLC-calibration solutions were prepared by transferring 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml aliquots of FA-21-PhP solution into 10 ml volumetric flask followed with 5 ml of ethanol, then 1.0 ml of sodium hydrogen carbonate stock aqueous solution was added and filled up to mark with ethanol. Chromatograms of each calibration solutions have been recorded immediately after preparation under the conditions cited in Section 2.2 (Section 2.2.2.). The detector wavelength was 238 nm. Full run time was 15 min. Peak area of FA-21-PhP was used for regression analysis. Limit of detection for FA-21-PhP was experimentally determined using the concentration 0.0539 mM.

2.5. Procedure for solvolysis sample mixture

Accurately weighted 20 mg of FA-21-PhP was quantitatively transferred into volumetric flask of 100 ml with ethanol (approximately volume 60 ml) for complete dissolution, then 10 ml of sodium hydrogen carbonate stock aqueous solution was added and the flask fill up to mark with ethanol. The prepared mixture was vigorously shaken and transferred into flask with magnetic stirrer. The speed of 200 rpm was selected at magnetic stirrer.

For DS analysis aliquots of solvolysis sample mixture were withdrawn at time intervals 2, 4, 6, 8 and 24 h and zero-order spectra were recorded under the same conditions as for calibration solutions. For each aliquot of sample mixtures, amplitudes $^2D_{274.96}$ were measured in the second-order spectra that correspond to FA content. The notation for amplitude measurements was accomplished according to a more generally applicable method [17].

For HPLC analysis 20 μl aliquots of same solvolysis sample mixtures were injected and chromatograms were recorded under the same conditions as for the calibration solutions. Retention time for FA-21-PhP peak was 10.95 min. The obtained peak area corresponds to FA-21-PhP content.

3. Results and discussion

3.1. Solvolysis of FA-21-PhP

The hydrolytic processes are important for corticosteroids monoester synthesis and it is also the pathway for conversion of corticosteroid-21-ester (prodrug) to active form. Ester prodrugs are hydrolyzed in vivo by esterases to give corresponding acids. In case of topical corticosteroids there is still a great need for versatile and simple processes, whereby esters could be hydrolyzed without heating or exposure to extreme pH values. Extreme conditions, such as the use of suitable alkoxydes, are not accepted for this class of compounds because of the presence of a relatively unstable dihydroxyacetone moiety [18].

The standard procedure, which applies a MeOH solution of alkali carbonates at low temperature [19], fails if the esters are sterically hindered. In contrast to this literature, new synthesized ester

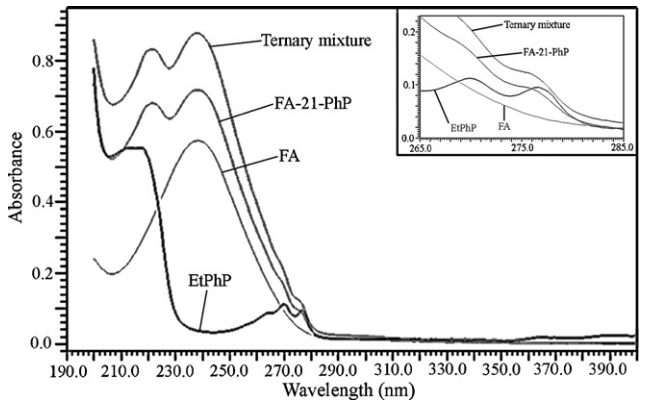


Fig. 2. The zero-order spectra of FA-21-PhP (61.5 μM), FA (38.3 μM), EtPhP (59.4 μM) and ternary mixture containing FA-21-PhP (56.3 μM) and equal concentrations of FA and EtPhP (10.7 μM). Inset: zoomed spectra in analytical λ range.

FA-21-PhP has a voluminous 2-phenoxypropanoic moiety which could solvolyse under the mild conditions due to steric effects with adjacent functional groups.

Under the selected conditions degradation product of FA-21-PhP is not 2-phenoxypropionic acid (PhPA) but it is ethyl ester (EtPhP) as a product of transesterification [20–24]. For this study synthesis of EtPhP has been carried out.

To select reaction conditions in relation to pH values, preliminary HPLC experiments were conducted using addition of aqueous solution of $NaHCO_3$ or phosphate buffer (pH 6.5) to ethanolic FA-21-PhP solution. Solvolysis percentage was lower in phosphate buffer, so further study was carried out in $NaHCO_3$ ethanol solution with mole ratio of FA-21-PhP to $NaHCO_3$ 1:20 (Scheme 2). HPLC-pilot experiments for mole ratio parameter (FA-21-PhP: $NaHCO_3$) have been performed to establish an approximate solvolysis percentage (24 h). In conditions with lower excess of $NaHCO_3$ the solvolytic reaction yields were lower than 50%. The extent of solvolysis decreases with increasing water content in reaction mixture. The quoted conditions have been used as in vitro model for activation of ester prodrug.

3.2. Spectral analysis

The zero-order spectra of parent ester (FA-21-PhP), both solvolytes (FA and EtPhP) and ternary mixture are presented in Fig. 2. The concentrations of separately recorded spectra of parent ester and solvolytic products are different in comparison to those of ternary mixture in order to show characteristics of each spectrum. The parent ester exhibits two characteristic absorption maxima and shoulders in wavelength range 260–280 nm. Zero-order spectra of solvolyte FA showed broad band with λ_{max} at 238.24 nm which corresponds to the first absorption maximum of FA-21-PhP at 237.76 nm. The absorption spectrum of solvolyte EtPhP is more complicated and those two small peaks in wavelength range 265–280 nm match with shoulders of parent ester. The absorption spectrum of ternary mixture exhibits previously mentioned λ_{max} and shoulders and the selected wavelength range 250–300 nm was used for monitoring solvolysis (inset spectra Fig. 2).

The selected initial concentration of parent ester for solvolysis analysis was five times higher in comparison to the one presented in Fig. 2, in order to monitor the process in analytical λ range (zommed spectra). Zero-crossing technique in derivative spectra is the most common procedure for the simultaneous determination of components in mixtures of their overlapping spectra. The fourth-order can discriminate FA in wavelength range 250–290 nm (Fig. 3) while mentioned shoulders or small peaks of FA-21-PhP and EtPhP respectively, give characteristic amplitudes (Fig. 3). In such

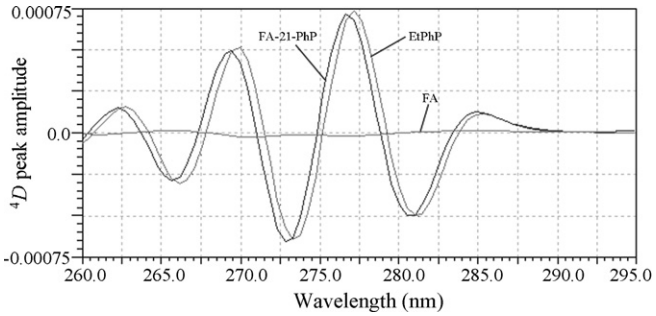


Fig. 3. Fourth-order derivative spectra of FA-21-PhP (0.200 mM), FA (0.125 mM) and EtPhP (0.185 mM).

way the solvolytic mixture using forth-derivative order has been transformed to binary mixture. Although, the zero-crossing points of parent ester and solvolyte EtPhP are at different wavelengths, but the difference is only 0.2 nm. Due to small values of amplitudes of fourth-order derivative spectra the precision could be considerably changed. Extremely small changes could significantly alter precision and accuracy of solvolysis percentages.

In the overlayed second-order derivative spectra of FA-21-PhP, FA and EtPhP, there is the only one joint zero-crossing point at 274.96 nm of FA-21-PhP and EtPhP (Fig. 4) which can be applied as analytical wavelength.

At this wavelength the amplitude $^2D_{274.96}$ of FA satellite corresponds only to FA content in solvolysis sample mixture (second-order derivative spectrum of real sample mixture with initial concentration of parent ester 0.335 mM after 6 h in Fig. 4). Lower sensitivity is due to amplitude $^2D_{274.96}$ of satellite peak. In general, this disadvantage of zero-crossing method in relation to sensitivity is due to necessity of selecting critical wavelengths as those of satellite peaks for measurements.

Other zero-crossing points of FA-21-PhP and EtPhP are at the different λ values with no analytical application. These findings confirmed that in certain cases DS cannot cope with the level of interferences especially when the spectra are strongly overlapped or in some cases of ternary mixtures.

3.2.1. DS method validation

The DS method validation was carried out according to ICH and USP regulative [25,26].

Determination of selected analyte in multi-component mixtures without previous separation in DS method enables the analysis by establishing appropriate derivative order, technique

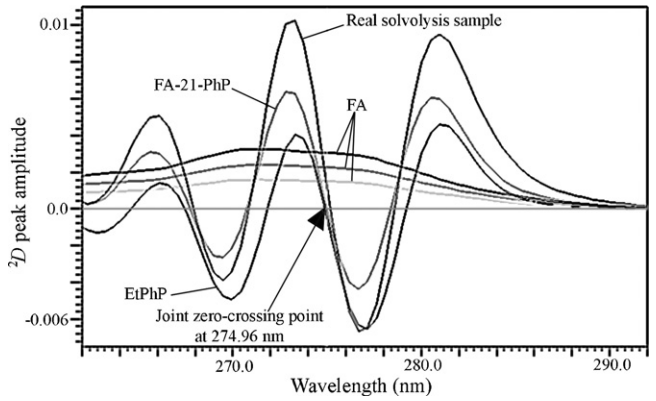


Fig. 4. Second-order derivative spectra of FA-21-PhP (0.200 mM), EtPhP (0.185 mM), FA (0.125, 0.187 and 0.250 mM) and real solvolysis sample mixture after 6 h (residual FA-21-PhP concentration 0.260 mM; obtained solvolytic products FA and EtPhP in equal concentrations of 0.075 mM).

Table 1
The results of solvolytic reaction of FA-21-PhP in three simultaneous real sample mixtures.

Time (h)	Confidence intervals (95%) for % of solvolysed FA-21-PhP		Solvolytic rate constant	
	HPLC	DS	HPLC	DS
2	8.76 ± 3.22	n.r.[a]	0.043 h^{-1} pseudo-first order	0.038 h^{-1} pseudo-first order
4	16.84 ± 3.05	12.11 ± 3.58		
6	23.47 ± 2.85	22.49 ± 3.25		
8	30.91 ± 2.68	33.99 ± 2.92		
24	64.81 ± 1.38	59.92 ± 1.90		

[a] n.r.: not reliable.

and value of smoothing points for specificity evaluation. The presence of FA-21-PhP and EtPhP are eliminated at joint zero-crossing point in second-order derivative spectra and solvolysis percentages were determined by increasing amplitude $^2D_{274.96}$ of solvolyte FA.

A linear relationship between amplitude $^2D_{274.96}$ and concentration was established in the range 0.062–0.312 mM. The regression equation was $y = 12.296[FA] + 0.00002$, the correlating coefficient being $r = 0.9997$, indicating excellent linearity. The experimental detection limit based on signal-to-noise ratio approach was obtained with 0.0312 mM. Experimentally obtained value of LOD (mM) defined as the analyte signal at $^2D_{274.96}$ which is three times higher in comparison to measured noise signal (in λ range 400–310 nm) was 0.0295 mM. The noise–signal (in λ range 300–250 nm) using blank solution was almost the same as noise–signal of sample solution (in λ range 400–310 nm). In such approach only one sample solution is required and from the same scan both analyte signal and noise–signal are measured and compared. In this way it is also possible to check simultaneously the effect of noise on analyte signal. Experimentally obtained value of LOQ (mM) defined at the analyte signal at $^2D_{274.96}$ which is ten times higher in comparison to measured noise signal (in λ range 400–310 nm) was 0.0983 mM.

For accuracy analysis ($n = 5$) the selected artificial solvolytic mixtures (20% of solvolysis corresponding to 0.27 mM of parent ester and 0.07 mM of each of solvolytic products) have been prepared. Those artificial solvolytic mixtures have been related to real sample after approximately 6 h of solvolysis process initiation. These mixtures have been spiked with three different concentrations of assayed FA. For added concentrations of FA 0.01, 0.05 and 0.1 mM corresponding recovery values were 98.25 ± 1.93, 101.00 ± 1.36 and 99.53 ± 0.88. The precision of the methods was measured only by means of repeatability while the inter-day precision was not possible to be evaluated since solvolysis has proceeded continuously in course of time. The RSD value for repeatability ($n = 6$) for FA concentration of 0.187 mM was 1.56%. To assess optimal conditions of DS method, the deliberate variation of smoothing point's values has been carried out. For the both adjacent smoothing point's values (15 or 19 in relation to optimal 17 points) overlaid spectra do not exhibit the joint zero-crossing point. The differences of 0.2 nm or 0.3 nm for 15 or 19 points, respectively in zero-crossing points of EtPhP and FA-21-PhP have been recorded. As it was expected for lower smoothing point's value the satellite amplitude of FA was distorted due to higher noise level along with lower amplitude value.

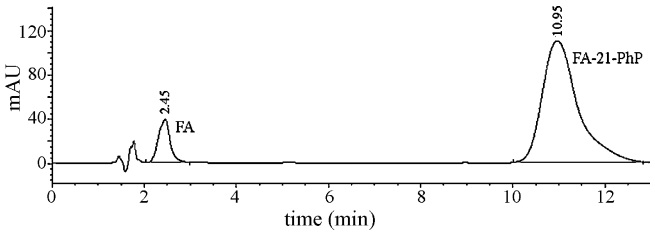


Fig. 5. Representative chromatogram of solvolysis sample mixture (after 2 h).

The concentration of parent ester (0.335 mM) in solvolysis sample mixture was selected in relation to lower absorptivity in analytical wavelength range. According to the regression equation, the concentration of FA was calculated on the basis of measured $^2D_{274.96}$ in each aliquot of solvolysis mixture. The calculated corresponding solvolysis percentages of FA-21-PhP are presented in Table 1.

3.3. Referent HPLC method

Representative chromatogram of solvolysis sample mixture is presented in Fig. 5. Only two components of the mixture could be monitored simultaneously at 238 nm, since solvolyte EtPhP at this λ has showed minimum. The retention times were 10.95 and 2.45 min of parent ester and FA, respectively, due to higher lipophilicity of ester. The solvolytic process was monitored using peak area of parent ester to ensure initial stage of changes in mixture. In order to apply HPLC as a referent method the same concentration of parent ester in solvolytic sample mixture was analyzed. For FA-21-PhP calibration curve, the presence of $NaHCO_3$ has the significant effect on parent ester peak shape (reducing tailing), so the comparison with peak area obtained from real samples of solvolytic mixtures is reliable. Since freshly prepared solutions have been recorded the peak areas correspond to the declared concentrations due to insignificant changes caused by solvolytic process during first 15 min.

A linear relationship between peak area and concentration was established in the range 0.054–0.540 mM. The regression equation was $y = 2 \times 10^7[FA\text{-}21\text{-}PhP] - 123.22$, the correlating coefficient being $r = 0.9994$, indicating excellent linearity.

The experimental detection limit based on signal-to-noise ratio approach was obtained with 0.0539 mM. Experimentally obtained value of LOD (mM) defined as the height of analyte signal which is three times higher in comparison to measured noise signal was 0.0041 mM. Experimentally obtained value of LOQ (mM) defined as the height of analyte signal which is ten times higher in comparison to measured noise signal was 0.0135 mM.

3.4. Kinetics of solvolysis

This study includes only the first part of experiments: possibility of application of second-order derivative spectrophotometry as well as the comparison with data obtained with HPLC assay of solvolytic reaction of FA-21-PhP. The achieved results could be used for further extensive studies of kinetics. In the assays of proposed DS and referent HPLC method signals of different compounds of ternary mixture were measured. In order to evaluate reaction order of solvolysis, it was essential to convert the results for FA in DS assay into concentration of remained FA-21-PhP, using percentages of solvolysis (Table 1). The straight lines were plotted using ln[FA-21-PhP] versus time (Fig. 6). These results were the average values of three sets of simultaneous real sample mixtures analy-

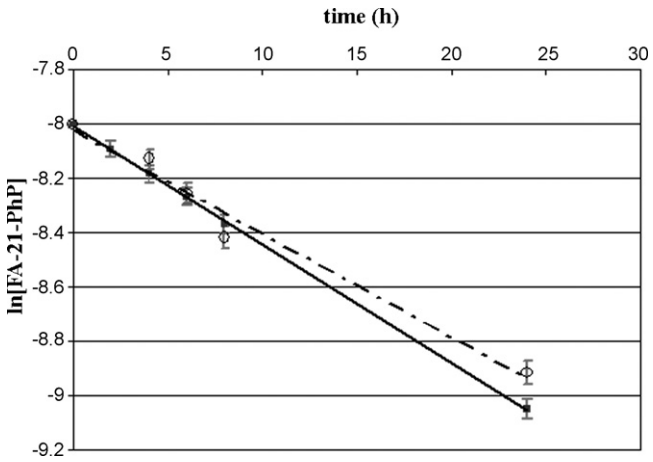


Fig. 6. Kinetic plots of FA-21-PhP solvolysis (0.335 mM) monitored by DS (- · -) and HPLC (—) with the marked bars of ln[FA-21-PhP] standard deviation; conditions ethanol:water (90:10, v/v) solution with mole ratio of FA-21-PhP to $NaHCO_3$ 1:20 at room temperature.

sis with final $NaHCO_3$ concentration of 6.7 mM (molar ratio 1:20 in relation to initial FA-21-PhP concentration). On the basis of linear relationship (Fig. 6) (for DS and HPLC correlation coefficients were 0.9891 and 0.9997, respectively) the reaction can be considered as a pseudo-first-order kinetics because it only depends on FA-21-PhP concentration when the concentration of $NaHCO_3$ is significantly higher. In accordance to obtained LOD values for FA-21-PhP in HPLC and for FA in DS the lowest solvolysis percentage in HPLC assay was about 1%, whereas in DS assay was about 8%. Percentage attained during first 2 h in DS assay was not reliable (Table 1) since it has been close to LOD value. The values of solvolysis rate constant obtained by DS and HPLC method are in good accordance (Table 1). The attained acceptable difference between solvolysis rate constants could be defined by using two different analytes for monitoring kinetic of solvolysis, FA as the product in DS method and FA-21-PhP as the parent ester in HPLC method, and also having in mind that DS method was performed without previous separation. The established results verified that simple DS method could be used as an alternative assay to HPLC method.

4. Conclusion

The presented study proved the effectiveness of UV-derivative spectrophotometry for monitoring of FA-21-PhP solvolysis. Due to the structural similarity of the solvolyte ethyl 2-phenoxypropionate (EtPhP) to the parent 2-phenoxypropionate ester of fluocinolone acetonide (FA-21-PhP), particular consideration was paid to the selection of derivative order for efficient spectra resolution in solvolysis of ternary mixture. The application of zero-crossing technique in second-order derivative spectrophotometry was possible at only one analytical wavelength (274.96 nm) which was the joint zero-crossing point of parent ester and solvolyte EtPhP. The second-order derivative assay of FA has a limitation in the initial stage of solvolysis due to lower sensitivity caused by means of FA amplitude satellite peak. It could be expected that derivative spectrophotometry (DS) in fourth-order spectra would be more efficient for in vivo analysis using esterases due to more substantial difference in zero-crossing points of parent ester and corresponding acid (PhPA). In this way FA is suppressed (binary mixture) and the sensitivity for parent ester assay would be higher due to central peak amplitudes measurements.

The HPLC method was used as a referent method and the peak area of parent ester (FA-21-PhP) was the signal for monitoring solvolytic reaction. Percentages of solvolysed FA-21-PhP, as well as the solvolytic rate constants, obtained by DS and HPLC confirmed that simple and fast DS method could be used as an alternative assay to HPLC method. DS method is efficient and offers high sample throughput in comparison with HPLC and therefore it certainly renders in-time data turnaround during multicomponent analysis and throughout monitoring of different reaction equilibria.

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