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Reverse phase HPLC for polar lipids. Simple and selective HPLC procedures for analysis of phospholipid-based derivatives of valproic acid and various non-steroidal anti-inflammatory drugs

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Abstract

DP-VPA (SPD 421) [1] is a new compound, where valproic acid is chemically bound to lecithin. It is a novel prodrug of valproic acid with targeted action for the treatment of epilepsy. RP-HPLC stability indicating method has been developed for this and relative compounds. Versatile detection techniques could be used with these LC procedures. The absence of non-volatile components in the mobile phase allows running the method with evaporative light scattering and MS-detectors. The method appeared to be sensitive, selective, reproducible, and stability indicating. It could be easily upgraded to bioanalytical procedures applying LC-MS technique. The method could be used as 'generic' for numerous compounds having similar design of the molecules, such as lecithin-based derivatives of diclofenac, naproxen, ibuprofen, and indometacin. The strategy of HPLC method development for polar lipids is discussed.

Keywords: HPLC; Reverse phase; Valproic acid; Phospholipids; Anti-epileptic drugs; Non-steroidal anti-inflammatory drug; Stability-indicating method; Bioanalysis

1. Introduction

DP-VPA (SPD 421) (Fig. 1) is a new compound, comprising valproic acid, chemically bound to phospholipid (lecithin). It is a novel prodrug of valproic acid with targeted action for the treatment of epilepsy [1], whose pharmaceutical action recently has been broadly investigated in comparison with other anti-epileptic agents [2–4].

In addition to DP-VPA, this core technology has been applied in the development of further novel compounds including lipid derivatives of non-steroidal anti-inflammatory drugs (DP-

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NSAID) (Fig. 2) useful for the treatment of diseases and disorders related to inflammatory conditions [5].

Some of these products comprise a mixture of homologues (1-palmitoyl- and 1-stearoyl-) in the ratio of 10:90–20:80 due to the composition of natural lecithin—a starting material for their synthesis.

These molecules may specifically release the active drug at the target site following cleavage by phospholipase A2 associated with excitatory or inflammatory processes. The DP-compounds are prominent for their noticeable targeting to the brain where they exhibit anti-epileptic, anti-inflammatory or neuroprotective effects.

Recently, the HPLC technique has become the most widely used in drug analysis, allowing development of selective and specific stability indicating assays, which are required by current pharmaceutical regulations.

HPLC analysis of lipids is a comparatively less developed field of application of this technique. Numerous methods, known from the literature, are mostly performed using normal phase chromatography, often require derivatization, and allow rather separation between different classes of lipids, than between individual compounds within a certain class [6,7].

The present paper summarizes the strategy and results of elaborating the reverse phase HPLC procedures for lecithin derivatives of drug substances, which should be used as analytical methods for pharmaceutical industry. These methods may also provide a basis for analytical development in the field of polar lipids.

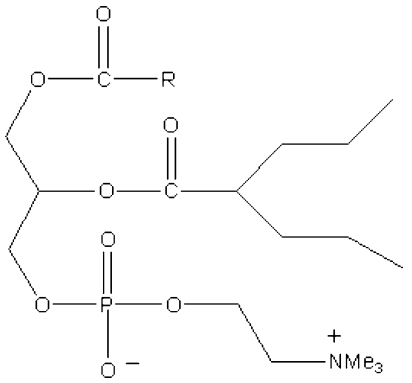

Fig. 1. DP-VPA (SPD 421): $R = C_{17}H_{35}$; $C_{15}H_{31}$.

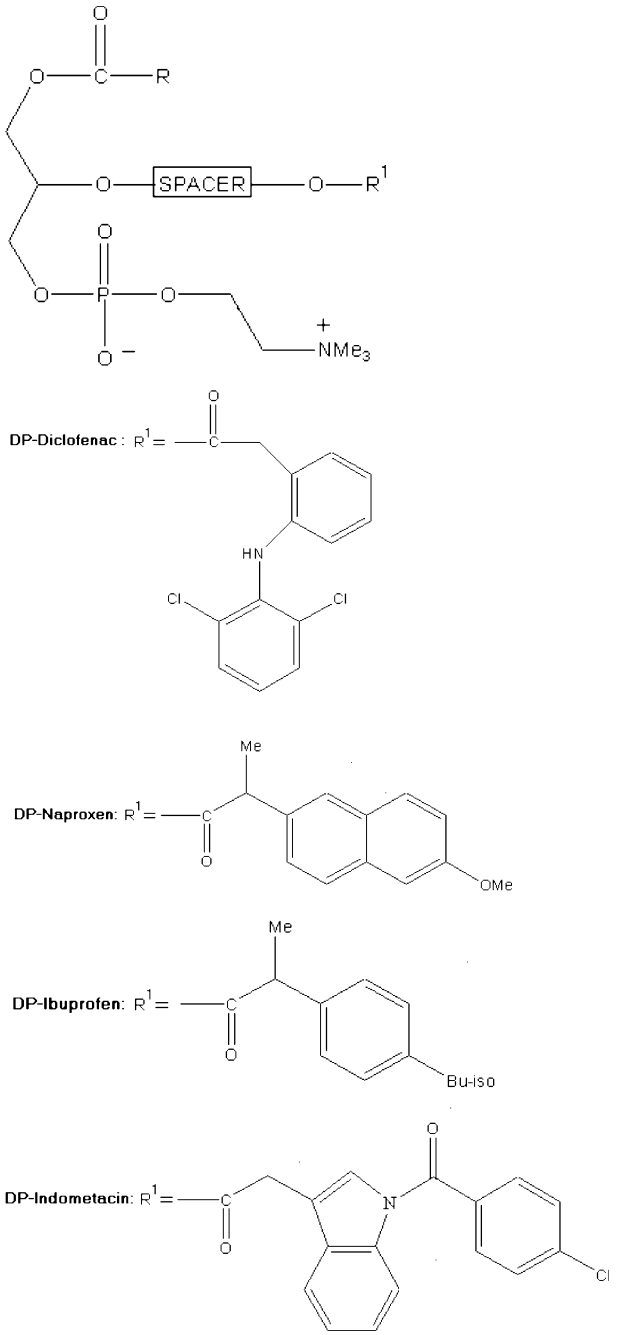

Fig. 2. DP-NSAID: $R = C_{17}H_{35}$; $C_{15}H_{31}$.

2. Experimental

2.1. Reagents and materials

DP-VPA, its ^{14}C-labelled analogue and lecithin derivatives of NSAID (diclofenac, naproxen, ibuprofen, and indometacin) were synthesized by the Chemical Department, D-Pharm Ltd. (Rehovot, Israel). Lysolecithin was purchased from Lipoid (Germany). Water, acetonitrile and methanol HPLC grade were obtained from BDH (UK) or Merck (Germany). Ammonium acetate reagent grade was purchased from Merck (Germany). Scintillation fluids Flo-Scint™ A or Flo-Scint™ M were provided by Packard Bioscience Company (USA).

2.2. Chromatographic instrumentation and equipment

LaChrom automatic HPLC system (Merck-Hitachi, Germany-Japan) consisting of L-7100 solvent delivery system, L-7200 autosampler, L-7400 multi-wavelength UV/Vis detector (set at 220 nm) and L-7000 interface.

Auxiliary detector for LaChrom system—the evaporative light scattering detector (ELSD) Sedex 55 (SEDERE, France).

Auxiliary detector for LaChrom system—the flow scintillation analyzer 150-TR (Packard, USA).

Alliance 2690 (or Alliance HS 2790) separation module automatic HPLC system (Waters, USA) equipped with UV/Vis photodiode array detector 996 (Waters, USA) and additional single quadrupole mass-spectrometric detector LC Platform with electrospray interface (Micromass, UK).

HPLC columns: Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2 (Agilent Technologies, USA); Inertsil® ODS2, 5 μm, 250 × 4.6 mm^2 (G.L. Sciences, Japan, or Teknokroma, Spain); Supelcosil™ RP-ABZ, 5 μm, 250 × 4.6 mm^2 (Supelco, USA); Symmetry® RP18, 3.5 μm, 50 × 2.1 mm^2 (Waters, USA); MonoChrom C18, 10 μm, 250 × 10.0 mm^2 (MetaChem, USA).

HPLC guard columns: OptiGuard C18 10 μm, 10 × 1.0 mm^2 (Optimize Technologies, USA).

2.3. Preparation of solutions

2.3.1. Solution preparation

DP-VPA and all its relative compounds were dissolved in methanol or acetonitrile to achieve the concentrations of 1–10 mg/ml.

2.3.2. Sample preparation for bioanalysis

To 0.5 g of tissue (brain, liver, intestine, etc.) 1 ml of 10 mM solution of ammonium acetate was added. The mixture was homogenized. One hundred microlitre of thus obtained homogenate was added to 200 μl of methanol, vigorously mixed and centrifuged (10 000 × g). The resulting supernatant was injected onto the chromatographic system.

To 20 μl of serum or plasma sample 200 μl of methanol was added, vigorously mixed and centrifuged (10 000 × g). The resulting supernatant was injected onto the chromatographic system.

2.3.3. Standard preparation for bioanalysis

To 1 g of tissue or 1 ml of serum or plasma 10 μl of methanol solution of DP-VPA, having the desired concentration, was added. For instance, using the solution of DP-VPA with concentration of 1 mg/ml, the standard concentration of DP-VPA will be 10 μg/g in tissues or 10 μg/ml in serum/plasma. For further processing the corresponding procedure for sample preparation was applied.

2.4. Chromatographic conditions

2.4.1. HPLC Conditions for analysis of DP-VPA as a chemical compound

Column	Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2, or Inertsil® ODS2, 5 μm, 250 × 4.6 mm^2, or Supelcosil™ RP-ABZ, 5 μm, 250 × 4.6 mm^2
Mobile phase	Methanol–acetonitrile–water (85:15:5 v/v)
Flow rate	1.0 ml/min
Detection	UV-detector, wavelength 220 nm, or ELSD, drying gas—air, pres-

	sure 3.5 bar, temperature 60 °C (mass-spectrometric detector—for structure elucidation)
Sample concentration	1.0 mg/ml
Injection volume	20 μl

2.4.2. HPLC conditions for bioanalysis of DP-VPA

Column	Symmetry® RP18, 3.5 μm, 50 × 2.1 mm^2
Mobile phase	Methanol–acetonitrile–water (88:11:1 v/v) with addition of ammonium acetate (0.7 g/l)
Flow rate	0.3 ml/min
Detection	MS detector, (electrospray) in positive ion mode, ion source temperature—+100 °C, cone voltage—+20 V
Injection volume	50 μl

2.4.3. HPLC conditions for bioanalysis of ^{14}C-labeled DP-VPA

Column	Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2
Mobile phase	Methanol–acetonitrile–water (85:15:5 v/v)
Flow rate	1.0 ml/min
Detection	Flow scintillation analyzer: setup for ^{14}C; scintillation fluid flow rate—1.0 ml/min; splitter'off'
Injection volume	20—50 μl

2.4.4. HPLC conditions for preparative separation of ^{14}C-labeled DP-VPA

Column	MonoChrom C18, 10 μm, 250 × 10.0 mm20—50^2
Mobile phase	Methanol–acetonitrile–water (85:15:5 v/v)
Flow rate	10.0 ml/min
Detection	Flow scintillation analyzer: setup for ^{14}C; scintillation fluid flow rate—1.0 ml/min; splitter: 2%—to detector, 98%—to fraction collector
Sample concentration	2.0 mg/ml
Injection volume	200 μl

3. Results and discussion

3.1. Development of HPLC method for DP-VPA (SPD 421)

DP-VPA, being a representative of lecithin-type substances, combines in one molecule a very polar zwitterion phosphatidyl choline moiety and lipophillic hydrocarbon chains of fatty acids. The marked ambiphilicity of the molecule of DP-VPA resulted the design of HPLC method development.

The initiation of chromatographic development for DP-VPA was the use of C18 or C8 columns in combination with mobile phases with a high content of organic solvent—acetonitrile and/or methanol, without buffer salts in aqueous part.

The 150-mm C18 columns were not efficient enough to achieve satisfactory resolution between the homologues (C16- and C18- DP-VPA) for reliable quantitative analytical procedure. The

columns C8 also did not provide satisfactory separation, probably due to their lower hydrophobicity. Application of 250-mm columns C18 resulted in acceptable separation and retention times for both C-16- and C-18- homologues of DP-VPA.

However, the 'classical' non-endcapped C8 or C18 columns did not provide well-shaped peaks. This could be due to the influence of silanol groups of the stationary phase, especially when the analyte molecule bears zwitterion moiety. Improving peak shape is usually achieved by adding ion-pair reagents. However, addition of non-volatile compounds to the mobile phase makes impossible using the evaporative detectors, such as light scattering and most types of mass-spectrometric. Taking into consideration the extremely weak chromophore of DP-VPA in the UV spectrum, this can cause limitations in sensitivity.

Therefore, the stationary phase of chromatographic column should keep away from silanol–zwitterion interaction without modifying the mobile phase. The stationary phase should be endcapped, or provide electrostatic or steric shielding of silanol groups.

Hence, the main criteria for the choice of HPLC column are:

1. Stationary phase should be based on highly purified or synthetic silica—it should be metal free. This condition takes into account the high tendency of ionic structures to irreversibly bind metal ions, thus disturbing elution from the chromatographic column.
2. Reverse phase column should be with endcapped or shielded silanol groups to avoid any residual silanol activity. This will also ensure high hydrophobicity of the stationary phase even for moderate carbon loading.
3. Separation should be carried out using comparatively low polar eluent, such as acetonitrile–methanol mixtures with low content of water, not containing buffer salts and ion-pair reagents.
4. The stationary phase should be highly hydrophobic.

The following stationary phases fulfill the above requirements:

- Phases with the polar group imbedded into the hydrocarbon chains, which prevents the silanol–analyte interactions due to electrostatic shielding of silanol groups. The examples: RP-ABZ and RP-ABZ + plus of Supelco, having imbedded amide group, or Symmetry Shield of Waters, with carbamino group imbedded.
- Phases with bulk side-groups in long-chained moieties attached to silica, which provide similar effect due to steric hindrance of silanol groups—masking or shielding effect. The example—Eclipse of Zorbax.
- Endcapped phases with 'tight packing' of long-chained organic modifier of silica, substantially reducing accessibility of remaining free silanol groups. The example—ODS-2 or ODS-3 (ODS-3V) of Inertsil, having high carbon loading and very regular structure of C18 chains on silica surface.

Sufficient separation (resolution factor 4) was reached using the column Supelcosil™ RP-ABZ, 5 μm, 250 × 4.6 mm^2 with the mobile phase: methanol–acetonitrile–water (85:15:5 v/v) and flow rate 1.0 ml/min (Fig. 3). Well-shaped symmetrical peaks for both homologues of DP-VPA were obtained.

However, the retention time using this column, was low. Therefore, there was a risk of missing the relative compounds, eluting earlier, such as lysolecithin, a starting material for synthesis of DP-VPA. More than baseline separation between the two homologues was achieved, but the difference in retention time was still low, causing concern about baseline separation from possible impurities, which could elute between the main peaks (for instance, the C17-homologue).

The usual approach in RP-chromatography—to increase the aqueous phase content in the eluent in order to increase retention time—does not work in this case. It causes only decline of performance and decrease of peak shape. Therefore, taking into account the comparatively low hydrophobicity of columns RP-ABZ, RP-ABZ + plus and Symmetry Shield, it was decided to turn from the columns with electrostatic shielding to those with steric one, known for their higher hydrophobicity.

The results obtained on the column Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2, using the same setup as for the Supelcosil™ LC-ABZ,

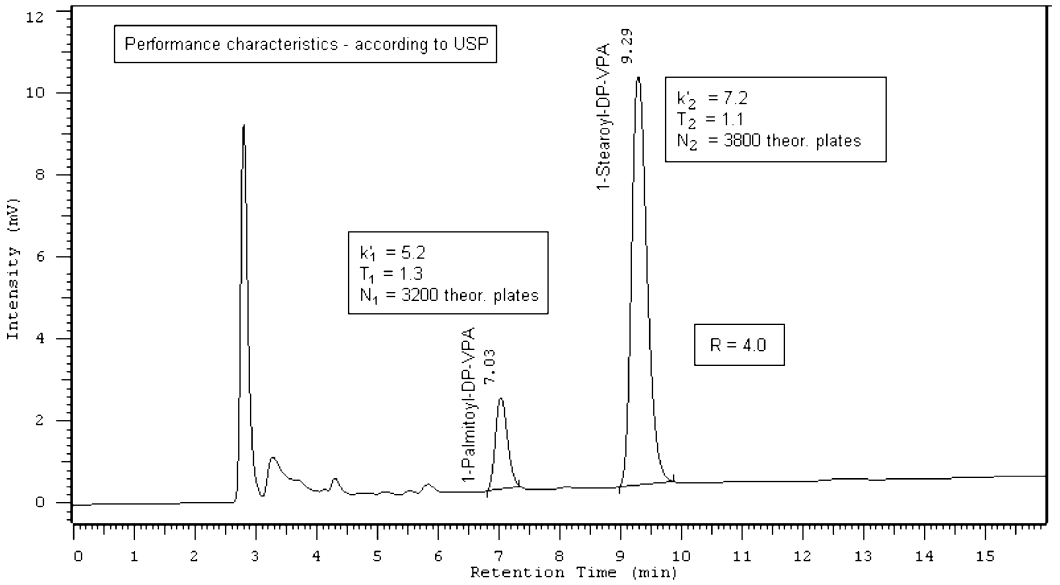

Fig. 3. Chromatogram of DP-VPA on the column Supelcosil™ RP-ABZ, 5 μm, 250 × 4.6 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v); flow rate 1.0 ml/min; detection—UV at 220 nm.

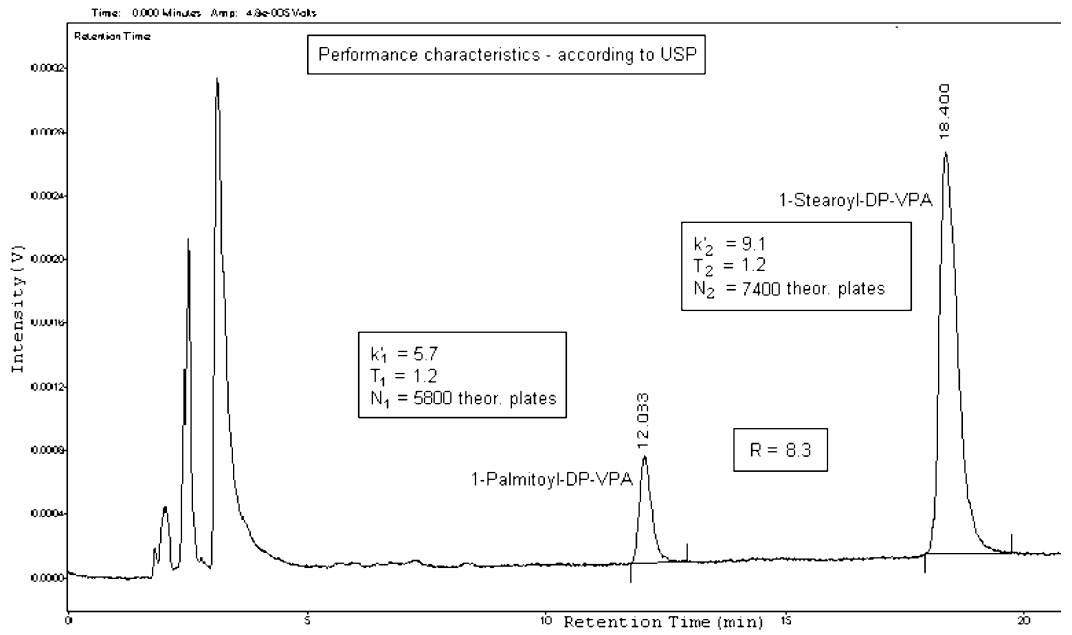

Fig. 4. Chromatogram of DP-VPA on the column Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v); flow rate 1.0 ml/min; detection—UV at 220 nm.

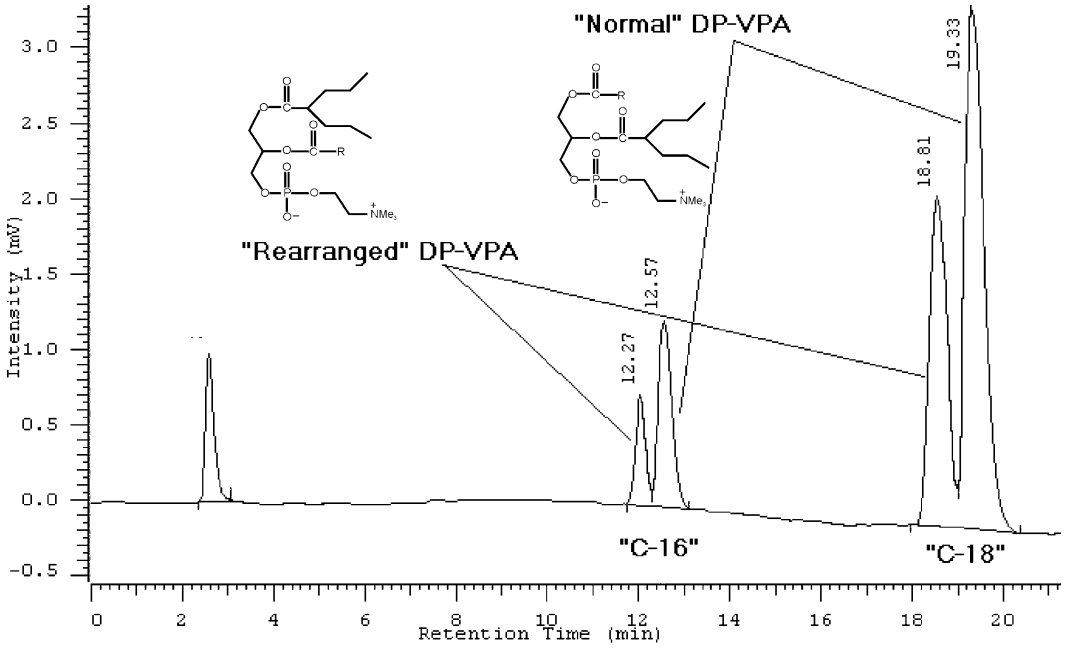

Fig. 5. Chromatogram of the mixture of isomers of DP-VPA.

allowed to develop a chromatographic procedure, which complies with all the modern requirements for HPLC methods.

The quality of chromatographic separation provides for clear resolution of even the close homologues within the same class of phospholipids. As could be seen from the chromatogram (Fig. 4), two compounds, differing by only two methylene groups, have well-shaped peaks, and the retention time difference between the peaks reaches 5 min.

Later it was found, that the column Zorbax Eclipse® XDB-C18 could be successfully substituted with Inertsil® ODS 2 or ODS 3 (ODS 3V), with the same particle size and dimensions—5 μm, 250 × 4.6 mm^2. These columns are characterized by very similar high hydrophobicity and low silanol activity as Zorbax Eclipse® XDB-C18. The chromatographic conditions are easily adjustable to obtain the same performance (peak shape, retention times, etc.), so that these columns can be considered as equivalent for this method.

The developed procedure does not require derivatization, thus providing the possibility of analyzing not only the main compounds, but also possible related compounds (both synthetic impurities and degradation products). This elevates the method to a stability indicating analytical procedure. Due to the weak chromophore, the analysis of related compounds (minor components of the analyte) requires 10-fold higher loading of the sample onto the HPLC column.

Moreover, the developed method appeared even to be capable of resolving the structure isomers, and is therefore applicable for recognition of the position isomers of DP-VPA, which could eventually be formed in the acylation reaction of lysolecithin (Fig. 5).

3.2. Validation of the developed chromatographic method

The developed chromatographic method, followed by UV detection at 220 nm, was validated in terms of precision, accuracy, linearity, sensitivity and robustness [8,9]. The method appeared to be precise, accurate and linear in the range of 0.2–2.0 mg/ml, complying with FDA and ICH requirements.

(1) *Precision:* RSD for 5 replicate injections of the DP-VPA solution having working concentration of about 1.0 mg/ml—0.7%.

(2) *Accuracy:* deviation of the obtained result for the DP-VPA solution having working concentration of about 1.0 mg/ml from the theoretical value—within $\pm 1.5\%$.

(3) *Linearity:* the method is linear in the range 0.5–2.5 mg/ml, $r^2 = 0.9980$.

(4) *Detection and quantitation limits:* (when applying 10-fold loading—10 mg/ml): 0.05 and 0.2% respectively.

(5) *Linearity for low concentrations:* the method is linear between 0.2% (quantitation limit) and 5.0% (upper limit of impurities), $r^2 = 0.9920$ (the percentage refers to the content of the main substance, for the solution having a concentration of 10 mg/ml).

(6) *Specificity:* for the samples, subjected to forced degradation conditions (heat, oxidation, exposure to light, acid and alkaline hydrolysis) no foreign matter was detected in the peaks of the main substance; at least baseline separation achieved between all the peaks of degradation products and between them and the peaks of the main substance. Therefore the method can be used as the stability indicating analytical procedure.

(7) *Robustness:* the analytical procedure is robust, and allows small but deliberate variations (within $\pm 10\%$) in the mobile phase composition, flow rate, column temperature. The method could be run on different types of HPLC instruments and applying various HPLC columns: Zorbax Eclipse® XDB-C18 and Inertsil® ODS 2 or ODS 3 (ODS 3V), with the same particle size and dimensions—5 μm, 250×4.6 mm^2.

3.3. Versatility of the developed chromatographic procedure

Due to the lack of strong chromophore in glycerophosphatidyl choline-type molecules, the sensitivity of the method, using UV-detection, is comparatively low. The necessity to work at low wavelength (220 nm) causes high baseline noise, and, therefore, provides an unpleasant signal-to-noise ratio. This could be considered as a method disadvantage, but the sensitivity can be substantially improved by using the alternative detection techniques, such as evaporative light scattering since the mobile phase does not include buffer salts (Fig. 6).

The absence of non-volatile components in the mobile phase allows to apply the method with MS-detectors, thus enhancing its selectivity and sensitivity (Figs. 7 and 8). To support ionization, thus providing the sensitivity gain, ammonium acetate was added to the mobile phase (0.7 g/l, with reference to the total mobile phase composition). This allows mass-spectral identification of the analyzed compounds, as well as their possible impurities, which could be formed during production or in the course of stability studies.

The possibility of mass-spectral detection makes the HPLC procedure applicable for pharmacokinetic and metabolic studies. Due to high specificity of mass-spectral detection for HPLC, it is possible to apply the general approach—to 'downgrade' the chromatographic part of the analytical procedure in order to achieve a fast method suitable for high throughput analysis.

Therefore, a narrowbore C18 column was used to develop an LC-MS bioanalytical method—Symmetry® RP18, 3.5 μm, 50×2.1 mm^2. This also required adjustment of chromatographic conditions, including eluent composition and flow rate. The mobile phase for this application became: methanol–acetonitrile–water (88:11:1 v/v), with addition of ammonium acetate (0.7 g/l), with flow rate decreased to 0.3 ml/min.

This method allowed to provide analytical support to various biological experiments with DP-VPA. To illustrate the possibilities of the developed bioanalytical method, a typical example of LC-MS chromatographic run of a rat serum sample from the pharmacokinetic study (26 h after intravenous administration of DP-VPA) is provided (Fig. 9). Both homologues of DP-VPA, as well as bis(tetradecanoyl)lecithin (internal standard), are detected under positive ion electrospray ionization on a single quadrupole instrument, using single ion record mode for the masses, corresponding to the protonated molecules ($M + 1$). Chromatographic run time reduced from 25 to 5 min, which allows high throughput. Limit of quantitation of this procedure is 0.1 ppm, and it could be substantially improved using a triple-quadrupole MS detector.

Pharmacokinetic and metabolic studies sometimes include the work with drug substances labeled with radioactive isotopes. The developed analytical procedure, in combination with flow scintillation analyzer as the HPLC detector, provides the analytical support for the studies with DP-VPA labeled with ^{32}P, ^{14}C or ^{3}H isotopes, characterized by β-radioactivity. The chromatographic procedure remains without any changes, as developed to be run with UV-detector.

Moreover, the analytical chromatographic procedure could be easily upgraded to preparative in order to purify the synthesized labeled DP-VPA, which is very expensive. For this purpose a semi-preparative HPLC column (MonoChrom C18, 10 μm, 250 × 10.0 mm^2) was used, and the flow rate increased to 10 ml/min. The mobile phase remained the same. This procedure was run for purification and separation of individual homologues of DP-VPA, labeled with ^{14}C on the carboxyl group of valproic acid. The flow after chromatographic column was splitted: 98% to fraction collector, and 2%—to the flow scintillation detector for monitoring (Fig. 10). Due to the absence of non-volatile components in the mobile phase, the recovery of the purified labeled material becomes simple.

3.4. Adjustment of the 'generic' HPLC method for analysis of DP-NSAID

The method, developed for DP-VPA, could be also adjusted for analysis of related compounds, having fatty acid moieties of different length, by the means of very simple variations of the ratio of mobile phase components. Complex mixtures of polar lipids containing numerous components with fatty acids widely differing in the hydrocar-

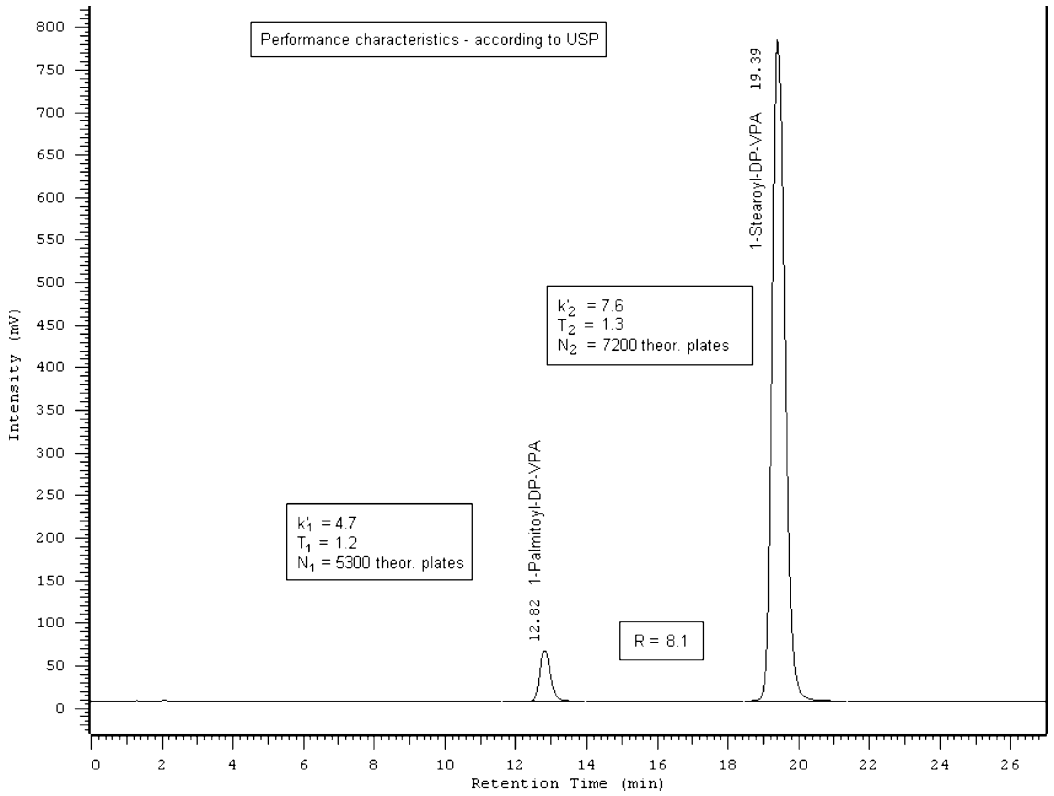

Fig. 6. Chromatogram of DP-VPA on the column Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v); flow rate 1.0 ml/min; detection—ELSD.

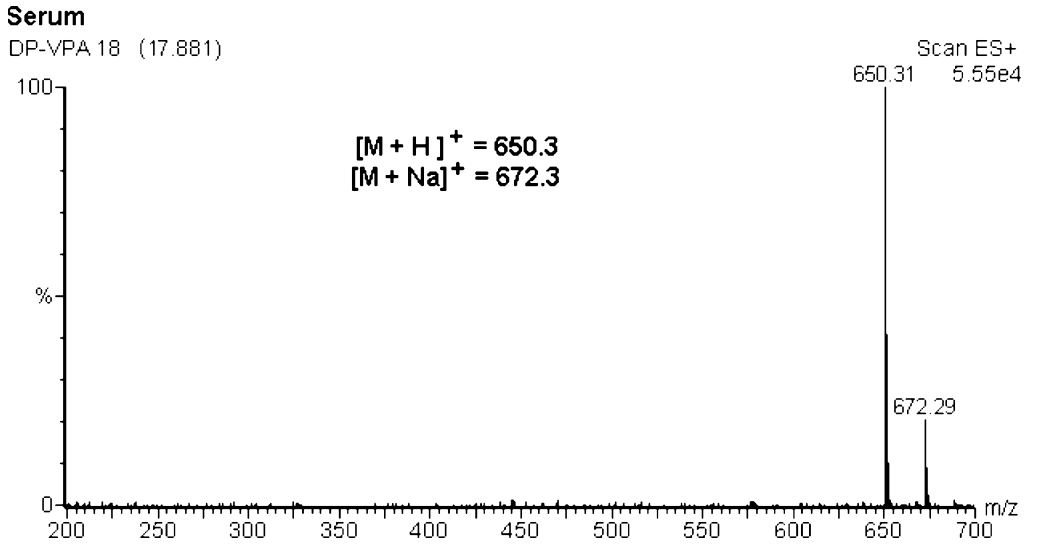

Fig. 7. MS of DP-VPA (C18) from the serum sample from LC-MS run on the column Zorbax Eclipse® XDB-C18, 5 μm, 250 × 4.6 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v) with 0.07% ammonium acetate; flow rate 1.0 ml/min; detection—MS (ES) scan in positive ion mode: 650.3 Da = $[M + H]^+$; 672.3 Da = $[M + Na]^+$.

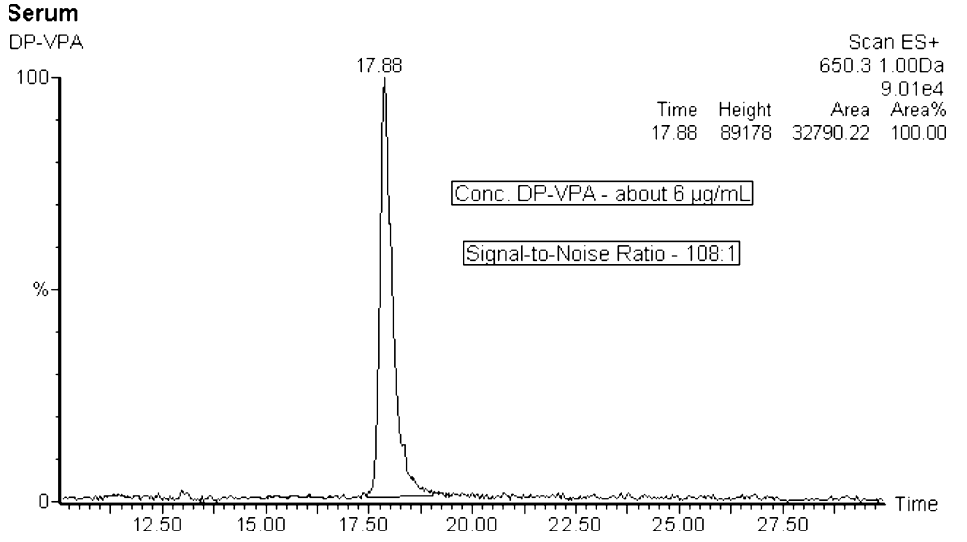

Fig. 8. Derived MS chromatogram of DP-VPA (C18) from the serum sample (for mass 650.3 Da, derived from the scan).

bon chain length could be analyzed applying simple gradients derived from the same 'generic' method.

In addition to analysis of DP-VPA, this analytical approach happens to be a good basis for development of generic analytical methods for similarly designed lipid derivatives of a number of drugs. A good example for this—easy adjustment of this method for analysis of lecithin-based derivatives of NSAID—diclofenac, naproxen, ibuprofen, and indometacin. This group of drug substances has been thus redesigned (DP-NSAID) for the treatment of various diseases [5].

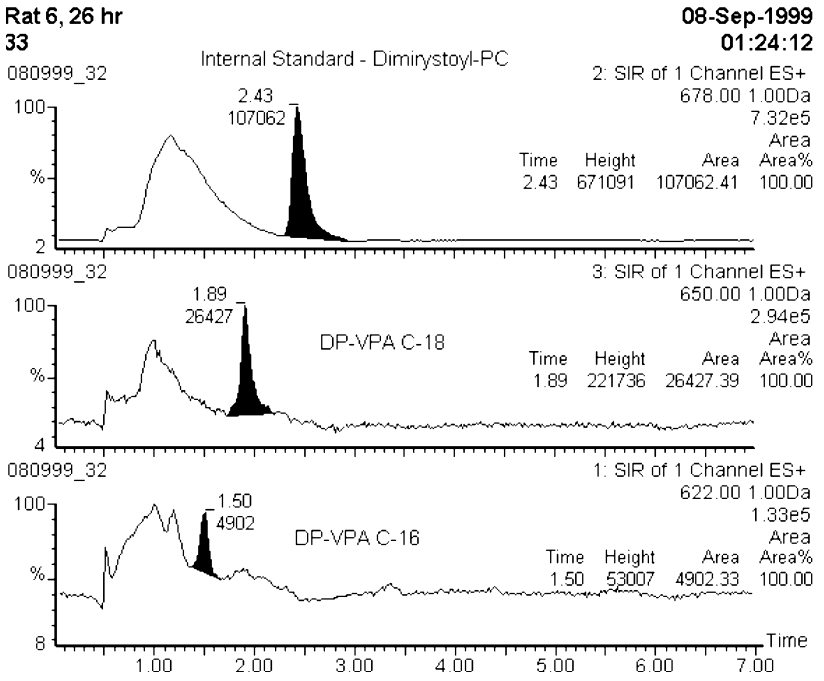

Fig. 9. LC-MS chromatogram of rat serum sample from pharmacokinetic studies on the column Symmetry® RP18, 3.5 μm, 50 × 2.1 mm^2; eluent methanol–acetonitrile–water (88:11:1 v/v) with 0.07% ammonium acetate, flow rate 0.3 ml/min; detection—MS (ES) single ion monitoring in positive ion mode.

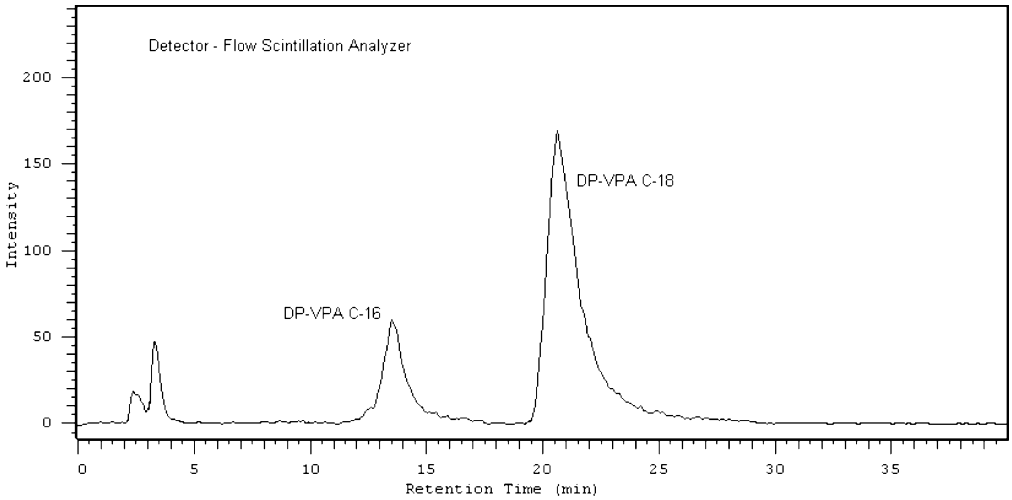

Fig. 10. Chromatogram of ^{14}C-labeled DP-VPA using flow scintillation analyzer: monitoring of semipreparative purification on the column MonoChrom C18, 10 μm, 250 × 10.0 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v); flow rate 10.0 ml/min; scintillation fluid flow rate—1.0 ml/min; flow split for detector—2:98.

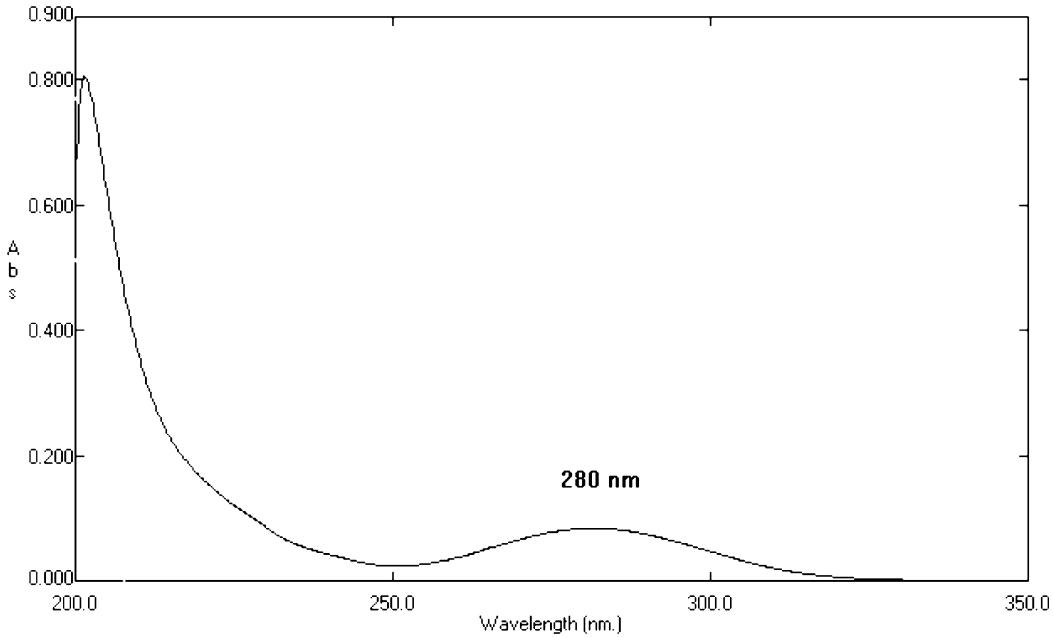

Fig. 11. UV-spectrum of DP-Diclofenac, concentration—7.8×10^{-7} mol/l solvent—mobile phase: methanol–acetonitrile–water (85: 15: 5 v/v).

From the point of view of the analyst, these substances are more 'friendly' than DP-VPA. All of them bear strong aromatic chromophores, and this facilitates detection by means of UV-detector and enhances sensitivity.

DP-Diclofenac is presented as an example of all the above DP-NSAID (Fig. 2). The starting point and general design of method development for three other substances (DP-Naproxen, DP-Ibuprofen, and DP-Indometacin) have much in common.

DP-Diclofenac, as well as diclofenac itself, has a strong characteristic UV-absorption at the wavelength 280 nm (Fig. 11).

Due to similar lipophillicity of all the DP-NSAID molecules and DP-VPA, there were almost no or slight adjustments of mobile phase composition. Chromatograms therefore look very similar to that for DP-VPA (Fig. 12). Actual retention time depends on the length of the 'spacer' moiety. Detection at comparatively high wavelength, such as 280 nm for DP-Diclofenac, substantially improved signal-to-noise ratio: DP-Diclofenac, having a concentration of 10 μg/ml (Fig. 12), has a higher signal-to-noise ratio, than DP-VPA with a concentration of 1 mg/ml (Fig. 4).

High sensitivity of the HPLC-UV procedure for DP-NSAID provides possibility to run this method for analysis of biological samples. For this purpose it is recommended to use the most sensitive UV-detectors, such as 2487 (Waters), or UV6000LP (ThermoFinnigan), which is equipped with a 50 mm flowcell.

4. Conclusions

- A simple and selective reverse-phase chromatographic procedure for a lecithin-based prodrug DP-VPA (SPD 421) has been developed and validated.
- The method could be used as generic for numerous compounds having similar design of the molecules, such as lecithin-bound NSAID diclofenac, naproxen, ibuprofen, and indometacin.

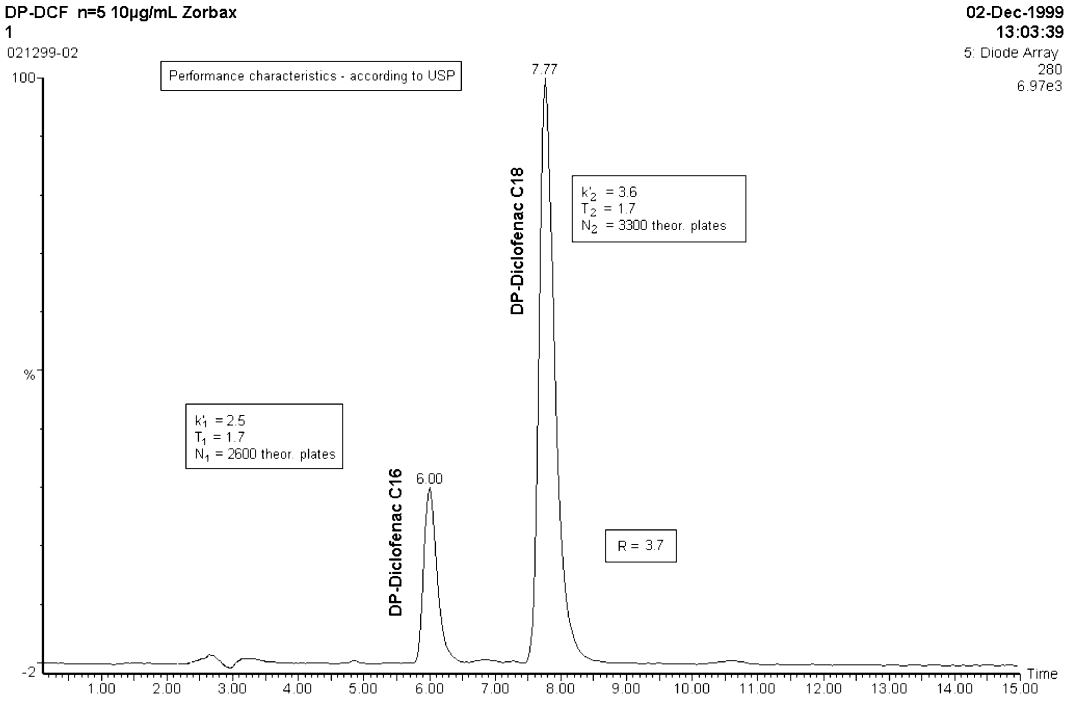

Fig. 12. Chromatogram of DP-Diclofenac on the column Zorbax Eclipse® XDB-C18, 5 µm, 250 × 4.6 mm^2; eluent methanol–acetonitrile–water (85:15:5 v/v); flow rate 1.0 ml/min; detection—UV at 280 nm.

- The developed method turns out to be a universal stability indicating procedure for a wide category of glycerophospholipids, requiring minimal adjustments.
- The method is easily adaptable for various detection techniques, including MS and ELSD, thus allowing sensitivity and specificity gain.
- In combination with flow scintillation analyzer, the method is applicable for analysis of the compounds, labeled with radioactive isotopes, and could be upgraded for their preparative purification and isolation.

References

[1] Prodrugs with enhanced penetration into cells. US Patent No. 6,166,089.
[2] M. Nei, J.M. Lee, V.L. Shanker, M.R. Sperling, Epilepsia 40 (2) (1999) 163.
[3] R.L. Kusty, D.F. Farrell, G.A. Ojemann, Epilepsia 40 (3) (1999) 266.
[4] K.K. Jain, Expert Opin. Investig. Drugs 9 (4) (2000) 840.
[5] Phospholipid derivatives of non-steroidal anti-inflammatory drugs. International (PCT) Patent Publication WO 00/31083.
[6] T. Shibamoto (Ed.), Lipid Chromatographic Analysis. In: Chromatographic Science Series, vol. 65, Marcel Dekker, Inc, 1994, p. 412.
[7] J.H.P. Tyman, M.H. Gordon (Eds.), Developments in the Analysis of Lipids, The Royal Society of Chemistry, Cambridge, UK, 1994, p. 206.
[8] FDA: Guidance for Industry. Analytical Procedures and Methods Validation, August 2000.
[9] ICH Q2B: Validation of Analytical Procedures: Methodology, May 1997.