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Advantages of application of UPLC in pharmaceutical analysis

Lucie Nováková, Ludmila Matysová, Petr Solich *

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

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Abstract

Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. UPLC chromatographic system is designed in a special way to withstand high system back-pressures. Special analytical columns UPLC Acquity UPLC BEH C_{18} packed with 1.7 μm particles are used in connection with this system.

The quality control analyses of four pharmaceutical formulations were transferred from HPLC to UPLC system. The results are compared for Triamcinolon cream containing trimacinolone acetonide, methylparaben, propylparaben and triamcinolone as degradation product, for Hydrocortison cream (hydrocortisone acetate, methylparaben, propylparaben and hydrocortisone degradation product), for Indomethacin gel (indomethacin and its degradation products 4-chlorobenzoic acid and 5-methoxy-2-methylindoleacetic acid) and for Estrogel gel (estradiol, methylparaben, propylparaben and estrone as degradation product).

The UPLC system allows shortening analysis time up to nine times comparing to the conventional system using 5 μm particle packed analytical columns. In comparison with 3 μm particle packed analytical columns analysis should be shortened about three times. The negative effect of particle decrease is back-pressure increase about nine times (versus 5 μm) or three times (versus 3 μm), respectively. The separation on UPLC is performed under very high pressures (up to 100 MPa is possible in UPLC system), but it has no negative influence on analytical column or other components of chromatographic system. Separation efficiency remains maintained or is even improved. Differences and SST parameters, advantages and disadvantages of UPLC are discussed.
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Keywords: UPLC; Acquity; Pharmaceutical analysis; Triamcinolone; Hydrocortisone; Indomethacin; Estradiol

1. Introduction

Ultra Performance Liquid Chromatography (UPLC) could be considered to be a new direction of liquid chromatography. UPLC, as its first producer Waters proclaims, means "speed, resolution and sensitivity" [1].

As it is very well known from Van Deemter equations, the efficiency of chromatographic process is proportional to particle size decrease. According his model describing band broadening, which describes relationship between height equivalent of theoretical plate (HETP) and linear velocity, one of the terms (path dependent term), is dependent on a diameter of particle packed into the analytical column. Smaller particle diameter can significantly reduce HETP which results in higher efficiency and the flatter profile of Van Deemter curve (Fig. 1). Consequently, the mobile phase flow-rate increase does not have negative influence to the efficiency as it could be observed at 10 or 5 μm particles [2–4]. The negative aspect of small particle packed columns used in HPLC is, however, high back-pressure generating.

In conventional HPLC the choice of particle size must be a compromise. The smaller is the particle size, the higher column back-pressure is occurring in the HPLC system. That could be a limitation of the use of such columns in HPLC systems. Small column diameters like 2.1 or 1.0 mm could also cause similar problems and disable their use under the conventional conditions. Throughout the history of HPLC there has been a trend to use smaller particles packing material. Due to the pressure limitation of conventional

* Corresponding author. Tel.: +420 49 5067294; fax: +420 49 5518718.
E-mail address: solich@faf.cuni.cz (P. Solich).

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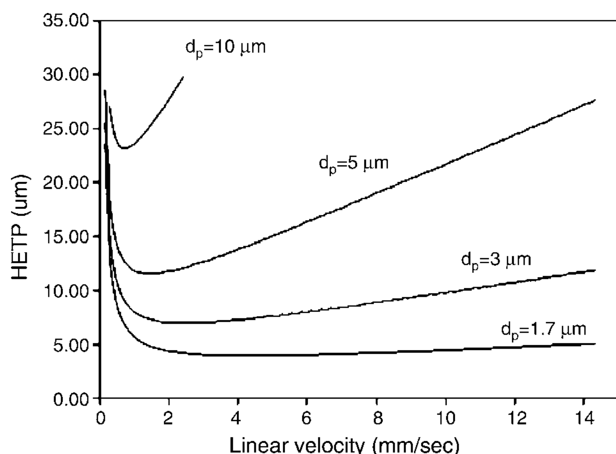

Fig. 1. Van Deemter curves for different particle sizes (10, 5, 3, 1.7 μm).

equipment, shorter columns packed with small particle diameter particles were used.

Several works dole with the development of ultra high pressure reverse phase liquid chromatography methods. MacNair et al. [5,6] have tested ultra high pressure liquid chromatographic system in connection with packed capillary columns using and 1.0 or 1.5 μm nonporous ODS-modified particles. They have also invented static-split injection technique, which was necessary to achieve high column efficiencies and withstand high pressures. Working pressure (496.8 MPa, 72,000 psi, respectively) used at their experiments was referred to be highest pressure used in liquid chromatography. The problems of their ultra high pressure experiments were possible thermal effects and pressure dependent retention effects. Forcing liquid through column with small particle packing could generate heat which had no chance to dissipate, especially at wide bore columns. The retained heat would significantly increase the temperature of mobile phase, the boiling point and in case of even smaller temperature changes danger of solute molecules decomposition occurred. Frictional heating and poor heat dissipation would also cause significant axial and radial temperature gradient. It was, however, discovered, that the heat dissipation problem could be overcome by reducing column diameter as described by Jorgenson and Lukacs for capillary electrophoresis [7]. The problem of heat generation was solved already by Halász et al. [8], who studied pressure limits and proposed to use 51.06 MPa (7400 psi) as the highest pressure limit in HPLC. The study refers also about fast separations using small particles. In this case 1 μm particles gave the fastest separation.

Another work by Wu et al. [9] tested ultra high pressure capillary liquid chromatography using fused silica capillaries packed with nonporous 1.5 μm isohexylsilane-modified (C_6) particles. The work discussed the aspect of injection and pumping system, it also stated that only capillary columns should be used in ultra high pressure liquid chromatography so as to facilitate frictional dissipation. Experimental pressure-balance injection valve was used for sample introduction and the comparison with previously described static-split injection was made. The effect of column diameter on efficiency and sensitivity in ultra high pressure chromatography was studied. The pressure-balanced injection system was found to be more convenient, reproducible and less sample requiring. Upper pressure limits allowed using maximum 100 MPa. The effect of column internal diameter on efficiency and sensitivity was found considerable.

Separation of chiral pharmaceuticals (including oxazepam, temazepam and chlortalidone) using ultra high pressure liquid chromatography was published by Xiang et al. [10]. Capillary columns containing C_6-modified silica particles of 1.0 μm size were used in connection with self-constructed ultra high pressure liquid chromatographic system described before [11]. UHPLC provided fast chiral separations (up to 2 min) with high resolution.

However, in order to use ultra high pressure chromatography routinely in the laboratory, some practical concerns, such as sample introduction, reproducibility and detection still needed an improvement. Ultra high pressure columns required extremely narrow sample plugs to minimize any sample volume contribution to peak broadening. To overcome these problems, Acquity UPLC system was developed because many of ultra high pressure systems used before needed in-house modification of commercial products by laboratory itself and also the own manufacturing of analytical columns [5,6,9] often capillary columns, as was stated above.

Acquity UPLC was specially designed as a first commercially available instrument so as to resist higher back-pressures than it was usual. While in conventional liquid chromatography the maximum back-pressure could be up to 35–40 MPa depending on particular instrument, in UPLC back-pressures could reach values up to 103.5 MPa (15,000 psi is given by specification). The system adjustments involve high pressure fluidic models like binary pump, which is able to work up to 15,000 psi as well as autosampler unit. Sample injection is characterized by fast injection cycles, low injection volumes, negligible carryover and temperature control (in a range 4–40 °C), which together contributes to the speed and sensitivity of UPLC analysis. Among the characteristics of detector, which utilize fibre optic flow cell with 10 mm pathlength and 500 nl cell volume belong high sampling rate, minimal dispersion and high acquisition rate (20–40 points/s). System volumes are minimized so as to keep speed, resolution and sensitivity of analysis [1,12].

The UPLC system is connected with specially designed Acquity UPLC columns containing X-Terra sorbent of second generation. The hybrid material utilizes bridged ethylsiloxane/silica hybrid (BEH) structure, particle size is only 1.7 μm. BEH technology ensures the column stability under the high pressure and through wider pH range (1–12) comparing to generation one X-Terra sorbent or

conventional stationary phases. Acquity UPLC columns are available with C_{18}, Shield RP_{18}, C_8 and Phenyl stationary phases [13].

The first practical applications of UPLC were carried out in connection with TOF mass spectrometry detection [14–19] on a field of metabonomics and genomic applications. The works showed explicit advantages of UPLC over HPLC in peak resolution together with increased speed and sensitivity on the field these fields. Recently, the first review on UPLC including theory of UPLC and summarizing some of the most recent work on the field has been published [20].

As efficiency and speed of analysis has become of a great importance in many application of liquid chromatography, especially on a field of pharmaceutical, toxicological and clinical analysis, where there it is important to increase throughput and reduce analysis costs, UPLC could play a significant role in the future of liquid chromatography.

The aim of this work was to make comparison between UPLC and HPLC analysis in pharmaceutical laboratory. Four complex topical formulations including Triamcinolon cream, Hydrocortison cream, Indomethacin gel and Estrogel gel were tested and results were compared.

2. Experimental

2.1. Chemicals and reagents

Working standards of active substances, preservatives and degradation products were used for the purposes of this study. Triamcinolone acetonide, hydrocortisone acetate and indomethacin (1-(4-chlorbenzoyl)-5-methoxy-2-methylindoleacetic acid) active substances were provided by Herbacos (Pardubice, Czech Republic). Estradiol active substance was purchased from Sigma–Aldrich (Prague, Czech Republic).

Preservatives methylparaben and propylparaben were obtained from Sigma–Aldrich (Prague, Czech Republic).

Compounds used as internal standards including hydrocortisone, dexamethasone and ketoprofen ((2-(3-benzoylphenyl) propionic acid) were obtained from Sigma–Aldrich (Prague, Czech Republic). Other internal standard compound hydrocortisone acetate was purchased from Herbacos (Pardubice, Czech Republic).

Degradation products trimacinolone, hydrocortisone, 4-chlorobenzoic acid, 5-methoxy-2-methylindoleacetic acid and estrone were provided by Sigma–Aldrich (Prague, Czech Republic). All these compounds were checked against European Pharmacopoeia CRS standards (Strasbourg, France).

All solvents used for analyses were HPLC grade. Methanol Chromasolv was provided by Sigma–Aldrich (Prague, Czech Republic). Acetonitrile HPLC grade was obtained from Sigma–Aldrich (Prague, Czech Republic). Phosphoric acid 85% p.a. was obtained from Merck (Darmstadt, Germany).

HPLC grade water was prepared by Milli-Q reverse osmosis Millipore (Bedford, MA, USA) and it meets European Pharmacopoeia requirements.

2.2. Chromatography

UPLC analyses were performed on Waters Acquity Ultra Performance Liquid Chromatographic system (Waters, Prague, Czech Republic) with PDA detector, cooling autosampler and column oven enabling temperature control of analytical column. Data were collected and processed by chromatographic software Empower. With this UPLC system special analytical column was connected. X-Terra sorbent of second generation packed into Acquity UPLC BEH C_{18} (2.1 mm × 50 mm, 1.7 μm) was used as a stationary phase. UPLC analyses utilized flow-rates in a range 0.50–0.60 ml min^{-1}. All analyses were performed at 25 °C (laboratory temperature). Two microliter were used as injection volume using partial loop mode for sample injection.

HPLC analyses were performed on Shimadzu LC-2010 C system (Shimadzu, Kyoto, Japan) with built-in UV–vis detector and with column oven enabling control of temperature. The auto-sampler was conditioned at 25 °C. Chromatographic software Class VP 6.13 was used for data collection and processing.

Tested compounds in Triamcinolon cream were separated on Supelco Discovery C18 analytical column (125 mm × 4.0 mm, 5 μm provided by Sigma–Aldrich, Prague, Czech Republic) using a mixture of acetonitrile and water (40:60, v/v) as a mobile phase in isocratic mode at flow-rate 0.6 ml min^{-1}, ambient temperature. Detection of compounds was accomplished at 240 nm. Ten microliter were injected into chromatographic system.

Analysis of Hydrocortison cream was performed on Discovery C18 analytical column (125 mm × 4.0 mm, 5 μm provided by Sigma–Aldrich, Prague, Czech Republic). Mobile phase was a mixture of methanol, acetonitrile, water (15:27:58, v/v/v) pumped isocraticaly at 0.8 ml min^{-1}. Separation was performed at ambient temperature. Detection of compounds was accomplished at 238 nm. Ten microliter were injected into chromatographic system.

For analysis of Indomethacin gel, Zorbax-Phenyl SB analytical column (75 mm × 4.6 mm, 3.5 μm, Agilent Technologies, Prague, Czech Republic) enabled separation of indomethacin and its two degradation products during 7.5 min at ambient temperature. Chromatography was performed using isocratic elution with binary mobile phase composed of acetonitrile and 0.2% phosphoric acid (50:50, v/v) at flow-rate 0.6 ml min^{-1}. UV detection of compounds was carried out at 237 nm. Five microliter were used for sample injection.

The separation of all components in Estrogel gel was performed on Supelco Discovery C18 (250 mm × 3.0 mm, 5 μm, Sigma–Aldrich, Prague, Czech Republic) analytical column. The mixture of acetonitrile, methanol and water (23:24:53, v/v/v) was used as a mobile phase at flow-rate 0.9 ml min^{-1}

using isocratic elution. UV detection was accomplished at 225 nm. Ten microliter of sample were injected into chromatographic system. The elevated temperature at 40 °C was necessary for satisfactory results.

2.3. Reference standard preparation

The stock solutions of internal standards were prepared in a similar way for all types of analyses. Fifty milligram of internal standard was dissolved in 100 ml of appropriate solvent. For analysis of Triamcinolon cream internal standard hydrocortisone, hydrocortisone acetate, respectively, was dissolved in 100 ml of acetonitrile. For analysis of Hydrocortison cream internal standard dexamethasone was dissolved in 100 ml acetonitrile too. Working solutions of internal standards were prepared by 50 times dilution of stock solutions of internal standards by acetonitrile.

The stock solutions of degradation products were prepared by dissolving 5.0 mg triamcinolone and hydrocortisone, respectively, in 100 ml of acetonitrile.

Reference standard solution for Triamcinolon cream analysis was prepared in 100 ml volumetric flask by dissolving of 2.50 mg of triamcinolone acetonide, 5.00 mg of methylparaben and 1.25 mg of propylparaben in acetonitrile. Finally, 2.0 ml of internal standard hydrocortisone stock solution and 10 ml of trimacinolone degradation product stock solution were added. The flask was filled up to the volume with acetonitrile. Other details could be seen in the article [21].

Reference standard solution for Hydrocortison cream analysis was prepared in 100 ml volumetric flask by dissolving of 25.0 mg of hydrocortisone acetate, 2.5 mg of methylparaben and 1.25 mg of propylparaben in acetonitrile. Finally, 2.0 ml of internal standard dexamethasone stock solution and 10 ml of hydrocortisone degradation product stock solution were added. Thereafter, the flask was made up to the volume with acetonitrile. Other necessary information could be found in the appropriate article [22].

Details of preparation procedures of standard solutions for analysis of Indomethacin gel and Estrogel gel are given elsewhere. Analysis of Indomethacin gel involved active substance indomethacin and its degradation products 4-chlorobenzoic acid and 5-methoxy-2-methylindoleacetic acid using ketoprofen as internal standard [23], analysis of Estrogel gel involved active substance estradiol, preservatives methylparaben and propylparaben and degradation product estrone [24]. Hydrocortisone was used as internal standard for quantitation.

2.4. SST data measurement

The samples of standard solutions were injected 10 times into the chromatographic system either UPLC or HPLC. Peak retention times and SST data were checked for all compounds using flow-rates appropriate for the analytical column and system as well. Thus, for Acquity UPLC system the flow-rate were in a range 0.50–0.60 ml min^{-1}. For HPLC analyses flow-rates ranged from 0.6 to 0.9 ml min^{-1}. Details

Table 1
System suitability data for Triamcinolon cream analysis

SST parameters	Triamcinolone	Methylparaben	Triamcinolone acetonide	IS-hydrocortisone acetate	Propylparaben
Theoretical plates					
HPLC	3397	3178	4163	Not determined	5462
UPLC	1080	2143	3604	4315	4959
HETP (μm)					
HPLC	36.80	58.33	36.68	Not determined	25.21
UPLC	46.30	23.33	13.87	11.59	10.08
Asymmetry factor					
HPLC	1.20	1.11	1.20	Not determined	0.98
UPLC	1.15	1.14	1.06	1.05	1.03
Resolution					
HPLC	–	4.47	4.74	Not determined	7.06
UPLC	–	2.86	5.57	3.51	1.69
Repeatability-t_r (% R.S.D.)					
HPLC	0.92	0.76	0.23	Not determined	0.25
UPLC	0.06	0.05	0.06	0.08	0.07
Repeatability-A (% R.S.D.)					
HPLC	0.93	0.68	0.25	Not determined	0.27
UPLC	1.13	0.34	0.29	0.56	0.48
Analysis duration (min)	HPLC		8.00		
6.9×	UPLC		1.15		
Solvent consumption (ml)	HPLC 0.6 ml min^{-1}		4.80		
8.3×	UPLC 0.5 ml min^{-1}		0.58		

Table 2
System suitability data for Hydrocortison cream analysis

SST parameters	Methylparaben	Hydrocortisone	IS-dexamethasone	Propyparaben	Hydrocortisone acetate
Theoretical plates					
HPLC	1607	1218	2091	4304	3478
UPLC	2211	3247	4198	4855	4674
HETP (μm)					
HPLC	77.78	102.63	59.78	29.04	26.74
UPLC	22.61	14.10	11.91	10.30	10.70
Asymmetry factor					
HPLC	1.02	1.18	1.12	1.04	1.01
UPLC	1.15	0.91	0.98	1.03	0.96
Resolution					
HPLC	4.82	2.92	3.30	2.41	2.23
UPLC	–	5.55	8.08	1.62	4.22
Repeatability-t_r (% R.S.D.)					
HPLC	0.16	0.18	0.23	0.17	0.19
UPLC	0.10	0.07	0.14	0.20	0.16
Repeatability-A (% R.S.D.)					
HPLC	0.44	0.41	0.25	0.31	0.28
UPLC	0.37	0.38	0.95	0.52	0.46
Analysis duration (min)	HPLC		11.00		
5×	UPLC		2.20		
Solvent consumption (ml)	HPLC 0.8 ml min^{-1}		8.80		
6.7×	UPLC 0.6 ml min^{-1}		1.32		

are given above and also in Tables 1–4. All analyses were performed at 25 °C, except of Estrogel gel analysis, which had to be performed at 40 °C. The mean values and R.S.D. of retention times and peak areas together with others SST parameters (theoretical plate number, peak asymmetry, peak resolution) were calculated for all components. The rules for measurement and the limits for the acceptance are given by appropriate guidelines [25,26] and pharmacopoeias [27,28].

Table 3
System suitability data for Indomethacin gel analysis

SST parameters	5-Methoxy-2-methylindoleacetic acid	4-Chlorobenzoic acid	IS-Ketoprofen	Indomethacin
Theoretical plates				
HPLC	3103	4000	5550	7655
UPLC	1673	2543	3520	5419
HETP (μm)				
HPLC	24.17	18.75	13.51	13.79
UPLC	29.89	19.66	14.20	9.23
Asymmetry factor				
HPLC	1.24	1.15	1.13	1.06
UPLC	1.14	1.07	1.03	0.98
Resolution				
HPLC	3.11	2.70	5.34	5.59
UPLC	–	3.53	3.59	11.33
Repeatability-t_r (% R.S.D.)				
HPLC	0.18	0.23	0.33	0.34
UPLC	0.10	0.04	0.01	0.12
Repeatability-A (% R.S.D.)				
HPLC	0.45	0.66	0.58	0.18
UPLC	0.73	0.45	0.63	0.34
Analysis duration (min)	HPLC		7.50	
4.7×	UPLC		1.60	
Solvent consumption (ml)	HPLC 0.6 ml min^{-1}		4.50	
5.6×	UPLC 0.5 ml min^{-1}		0.80	

Table 4
System suitability data for Estrogel gel analysis

SST parameters	Methylparaben	IS-hydrocortisone	Propylparaben	Estradiol	Estrone
Theoretical plates					
HPLC	2772	3274	5438	6015	7430
UPLC	1914	3484	5508	6190	6819
HETP (μm)					
HPLC	90.19	76.36	45.97	41.56	33.65
UPLC	26.13	14.35	9.08	8.08	7.33
Asymmetry factor					
HPLC	1.30	1.14	1.28	1.23	1.34
UPLC	Did not count	1.12	1.02	1.00	1.00
Resolution					
HPLC	–	4.99	6.86	6.12	4.24
UPLC	–	5.75	6.73	7.61	4.56
Repeatability-t_r (% R.S.D.)					
HPLC	0.00	0.00	0.00	0.09	0.10
UPLC	0.07	0.15	0.15	0.24	0.25
Repeatability-A (% R.S.D.)					
HPLC	0.19	0.16	0.18	0.14	0.10
UPLC	0.73	0.72	0.53	0.73	0.85
Analysis duration (min)	HPLC		12.00		
5.2×	UPLC		2.30		
Solvent consumption (ml)	HPLC 0.9 ml min^{-1}		10.80		
8.5×	UPLC 0.55 ml min^{-1}		1.27		

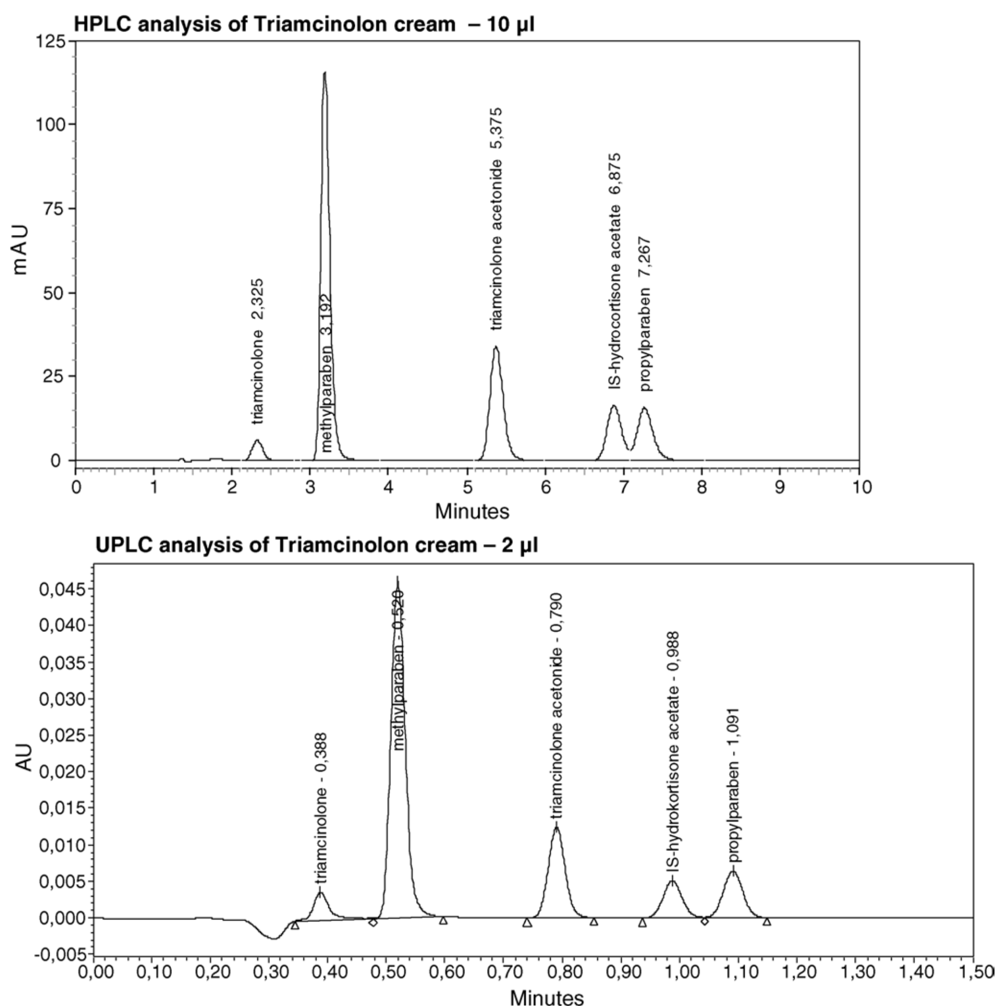

Fig. 2. Comparison of HPLC and UPLC analysis for Triamcinolon cream analysis–triamcinolone (0.5 mg/100 ml), hydrocortisone acetate–IS (1.0 mg/100 ml), methylparaben (2.5 mg/100 ml), triamcinolone acetonide (2.5 mg/100 ml), propylparaben (1.25 mg/100 ml).

3. Results and discussion

3.1. Triamcinolon cream

The method developed and validated for Trimacinolon cream analysis belongs among the oldest in our laboratory. It has been used for 5 years for routine determination and quantitation of active substance, preservatives and degradation product in pharmaceutical formulation during manufacturing process and stability studies. The method was simple, using isocratic elution by binary mobile phase acetonitrile and water (40:60, v/v) for separation of all tested compounds on conventional octadecylsilica column. Recently, after its transfer from older instrument to newer fully automatic instrument (Shimadzu LC 2010); however, problems with separation of internal standard occurred. Originally hydrocortisone was used as internal standard. The compound eluted at similar retention time as methylparaben, but the separation was satisfactory [14]. After the transfer between two instruments unfortunately coelution of methylparaben and hydrocortisone were observed. Therefore, hydrocortisone acetate was tested to be an internal standard instead of hydrocortisone. Neither in the case of hydrocortisone nor in the case of hydrocortisone acetate separation was found to be sufficient as it could be seen in Fig. 2—HPLC separation. In this case, UPLC with its higher efficiency was very helpful Fig. 2—UPLC separation because the system was able to provide separation which met validation requirements. Moreover, the transfer of this method into UPLC needed only mobile phase flow-rate change and injection volume decrease. That was very convenient, taking into mind that transfer between two HPLC systems did not work well.

Results of SST parameters could be compared in Table 1. UPLC analysis has been performed almost seven times faster than HPLC. Solvent consumption was decreased about eight times. The separation efficiency in HPLC was somewhat better for early eluting peaks, while in UPLC it was better for lately eluting compounds. In general, the values of HETP

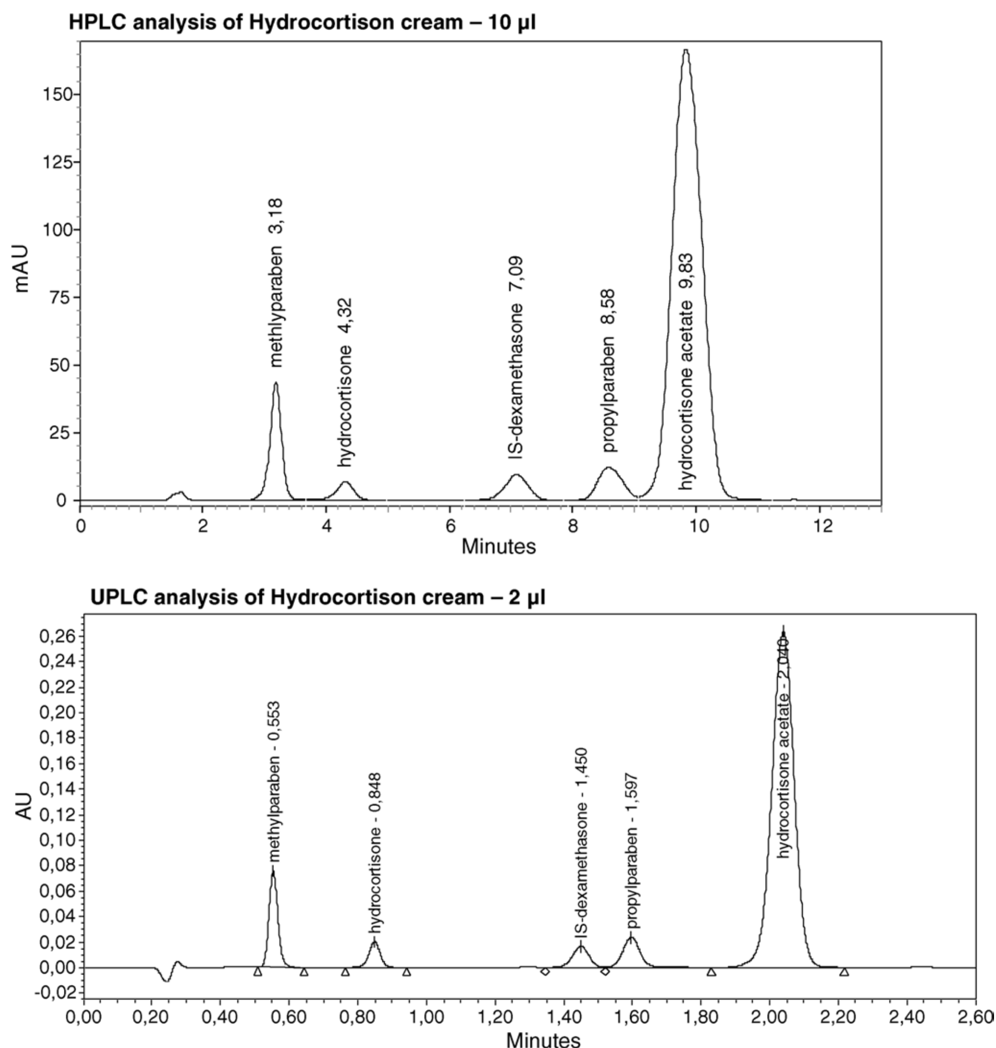

Fig. 3. Comparison of HPLC and UPLC analysis for Hydrocortison cream analysis–methylparaben (2.5 mg/100 ml), hydrocortisone (0.5 mg/100 ml), dexamethasone–IS (1 mg/100 ml), propylparaben (1.25 mg/100 ml), hydrocortisone acetate (25 mg/100 ml).

were comparable for both LC systems. Values of symmetry factor were comparable as well. Resolution of critical pair of peaks internal standard hydrocortisone acetate and propylparaben was sufficient only using UPLC method.

Retention time repeatability R.S.D. values were a little bit better in UPLC analysis. Peak area repeatability R.S.D. values were comparable for both chromatographic methods. That means also very good sensitivity of UPLC system because 2 μl injection volume of the same concentration was sufficient for reliable analysis results and integration.

3.2. Hydrocortison cream

The HPLC method used for Hydrocortisone cream analysis was originally developed and validated on fully automatic HPLC system about 3 years ago. In this case, however, the method probably was more robust ant its transfer to the newer and fully automatic HPLC system did not bring any problems. The transfer into UPLC method needed only mobile phase flow-rate change and injection volume decrease.

Hydrocortison cream analysis time was shortened five times in comparison with common HPLC method, as it could be seen in Fig. 3. This way solvent consumption per one analysis was reduced almost seven times. SST parameters are demonstrated in Table 2. In case of Hydrocortison cream analysis UPLC has shown much higher efficiency for all compounds. The highest difference was observed for hydrocortisone degradation product (7.28 times higher than HPLC) and the lowest difference in efficiency gave hydrocortisone acetate active substance (2.50 times higher at UPLC). Peak resolution was mostly higher in UPLC analysis except of pair of peaks propylparaben—dexamethasone.

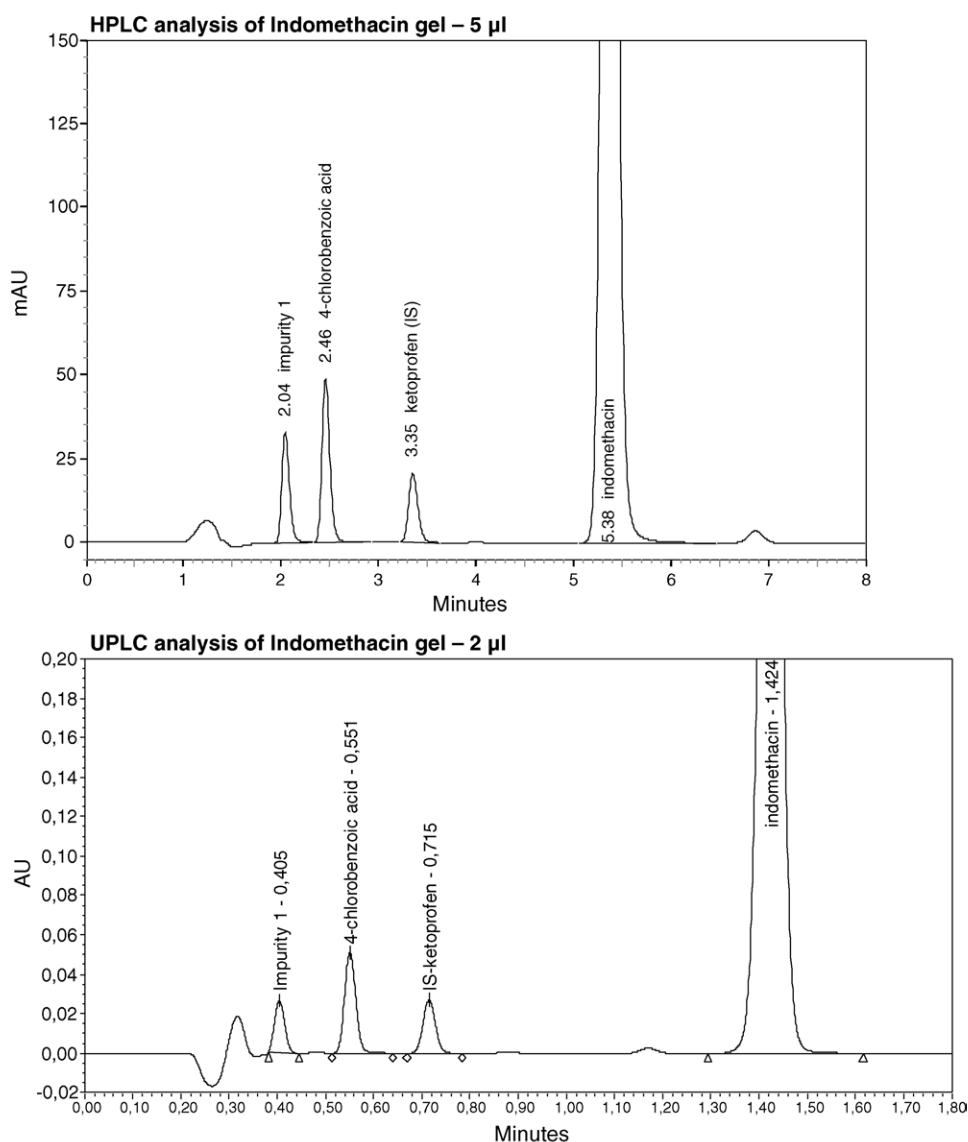

Fig. 4. Comparison of HPLC and UPLC analysis for Indomethacin gel analysis–impurity 1 = 5-methoxy-2-methylindoleacetic acid (0.5 mg/100 ml), 4-chlorobenzoic acid (0.5 mg/100 ml), ketoprofen–IS (1mg/100 ml), indomethacin (25 mg/100 ml).

Peak asymmetry values were very similar for both LC techniques.

Retention time repeatability was better for early eluting peaks in HPLC, while for lately eluting peaks the R.S.D. values were comparable. Peak area repeatability R.S.D. values were contrariwise, comparable for early eluting peaks, while for lately eluting peaks HPLC method gave better values of R.S.D.

3.3. Indomethacin gel

Analytical method for Indomethacin gel analysis was developed a couple of years ago for analytical evaluation of new developed pharmaceutical formulation and for its stability studies control. In method development new kinds of stationary phases were tested [23], it follows new trend in liquid chromatography of using smaller diameter particles. The best results gave Zorbax Phenyl SB analytical column (75 mm × 4.6 mm, 3.5 μm). As it could be seen, smaller particles and shorter column length was chosen for shortening analysis time and efficiency improvement. Method transfer into UPLC needed only decrease of injection volume and mobile phase flow-rate. No other changes were necessary.

In comparison with UPLC the analysis time was shortened 4.7 times, which was very good taking into mind 3.5 μm used particles, the confrontation could be seen in Fig. 4. Solvent consumption was decreased more than five times. SST parameters of UPLC and HPLC analyses (Table 3) were comparable for efficiency (HETP values), asymmetry factor and resolution as well. The same phenomenon was observed following comparison of HETP values—UPLC gave little bit worse efficiencies for early eluting peaks and little bit better efficiencies for lately eluting peaks.

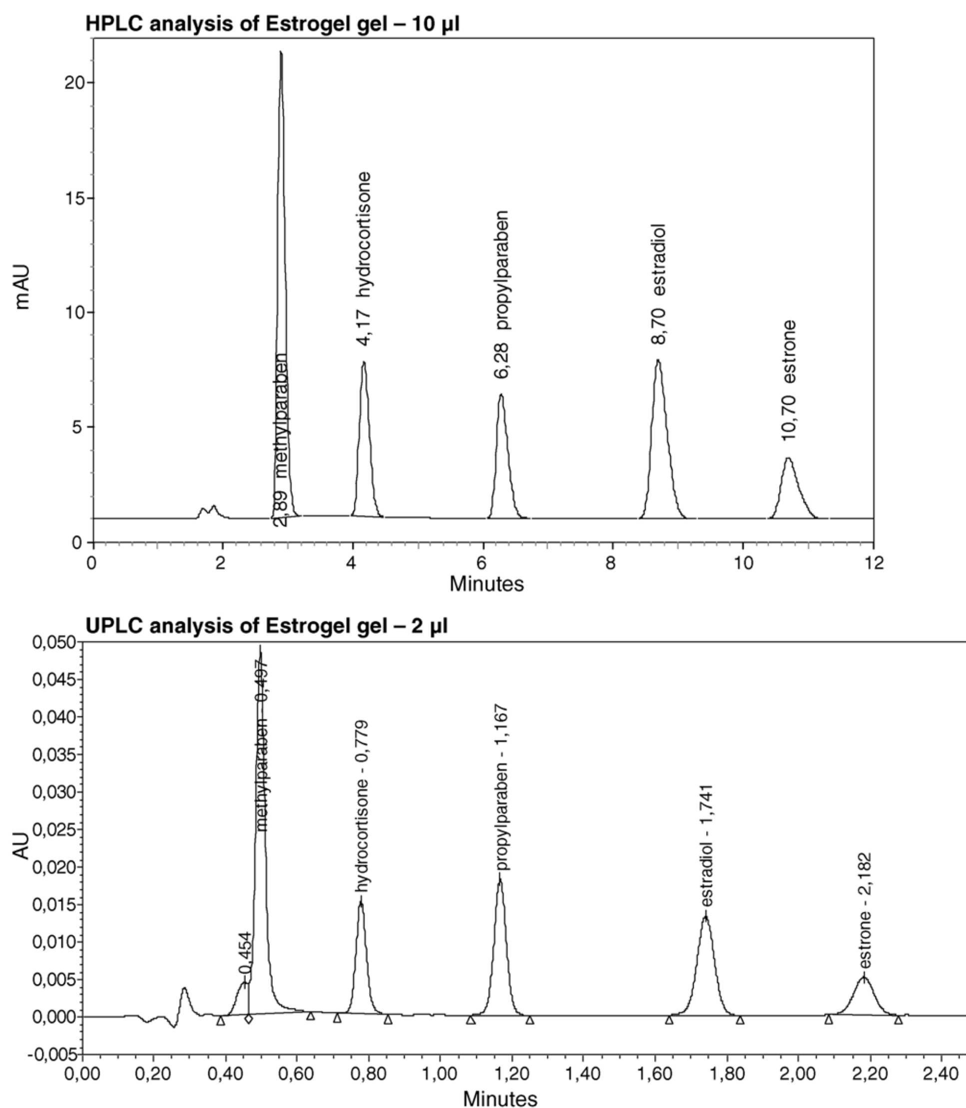

Fig. 5. Comparison of HPLC and UPLC analysis for Estrogel gel analysis–methylparaben (2.5 mg/100 ml), hydrocortisone–IS (1 mg/ 100 ml), propylparaben (1.25 mg/100 ml), estradiol (1.5 mg/100 ml), estrone (0.5 mg/100 ml).

Retention time repeatability R.S.D. values were better using UPLC method, peak area repeatability R.S.D. values were little bit worse in case of UPLC. The sensitivity of UPLC system was very good even using 2 μl of the same concentration as injection volume comparing to 5 μl injected in HPLC analysis. It could be said the results were almost the same in both chromatograms, even if one is depicted in mAU (HPLC) and next one in AU (UPLC).

3.4. Estrogel gel

Evaluation of pharmaceutical formulation Estrogel gel in our laboratory has taken about 2 years. The separation of estradiol and its degradation product was not easy, therefore long analytical column and increased analysis temperature was needed so as to reach sufficient compounds resolution. Method transfer into UPLC included temperature decrease (original 40 °C was not necessary), decrease in injection volume and also in mobile phase flow-rate.

Analysis duration was shortened more than five times due to UPLC. The comparison of chromatograms could be seen in Fig. 5. This way solvent consumption was decreased 8.5 times. In Estrogel gel analysis UPLC system had much better efficiency than common HPLC, except of methylparaben (3.4 times) it was about 5 times better for all compounds. Higher efficiency of UPLC system was also demonstrated by appearance of impurity peak eluting close to the peak of methylparaben. This impurity have not been observed or identified anytime before, even though the analysis of Estrogel gel was performed also on various analytical columns.

The values of asymmetry factor for tested compounds were better in UPLC analysis, especially for lately eluted peaks. Resolution of individual compounds remained approximately the same. The results of SST measurements could be seen in Table 4.

Retention time repeatability R.S.D. values in this case were much better for HPLC analysis, as were also peak area repeatability R.S.D. values. The sensitivity of UPLC system was very good even for such low injection volumes as 2 μl. The detector response in mAU (Shimadzu LC 2010–25 mAU) versus AU (Acquity UPLC–0.050 AU) was two times lower.

4. Conclusion

The new type of liquid chromatography—Ultra Performance Liquid Chromatography was tested. The comparison of data was made for four pharmaceutical analytical methods transferred between HPLC and UPLC.

UPLC advantages are clearly obvious. The separation mechanisms is still the same, chromatographic principles are maintained while speed, sensitivity and resolution is improved. This all supports easier method transfer from HPLC to UPLC and its revalidation.

The main advantage was particularly a significant reduction of analysis time, which meant also reduction in solvent consumption. Our experiments showed 4.7–6.9 times analysis shortening, while solvent consumption decreased 5.6–8.5 times. From this point of view, UPLC is more convenient for complex analytical determination of pharmaceutical preparations. Analysis duration, solvent consumption and consequently analysis cost is a very important aspect in many analytical laboratories. Moreover, the time spent with new method optimisation is saved. The time needed for method development experiments, for column equilibration or re-equilibration while using gradient elution and for method validation is much shorter.

The comparison of efficiency was apparently seen from the height equivalent of theoretical plate values. As columns had different lengths, theoretical plate number was not sufficient for correct comparison. It could be seen, that Acquity UPLC analytical system had comparable efficiency for most analyses as commonly used HPLC. In case of Estrogel gel and Hydrocortison cream analysis the efficiency was even much better (about five times, about 3–7 times, respectively) in case of UPLC system.

Other SST data including peak asymmetry and peak resolution values were comparable altogether, all of them met required criterions. Only in case of Trimacinolon cream analysis UPLC system was necessary for separation of critical pair of peaks because HPLC separation power was not sufficient. In case of Estrogel gel analysis the peak asymmetry for lately eluting peaks was observed to be better in case of UPLC.

We could observe a little bit worse peak area repeatability values in UPLC analyses. This could be induced by small volumes injection or using "partial loop injection" mode, which is theoretically a little bit less precise comparing to full loop mode. Higher sample dilution and comparison of these two injection modes could give confirmation of this presumption.

Another possible negative aspect of UPLC could be high working pressure routinely used for analyses. As convention is to work up to 35–40 MPa in HPLC, considering more than 35 MPa to have negative influence to column lifetime, 100 MPa could be unimaginably too much for conservative users. Any negative phenomenon connected with these high pressures was not observed. It would take probably some years with a lot of practical experiments to evaluate this possible drawback.

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