Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 833–838

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Validated UPLC method for the fast and sensitive determination of steroid residues in support of cleaning validation in formulation area

Szabolcs Fekete[a,*], Jenő Fekete[b], Katalin Ganzler[a]

[a] Formulation Development, Chemical Works of Gedeon Richter Plc, Budapest X, Gyömrői út 19-21, Hungary
[b] Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Budapest, Szt. Gellért tér 4, 1111 Hungary

ARTICLE INFO

Article history:
Received 25 October 2008
Received in revised form 8 December 2008
Accepted 15 December 2008
Available online 27 December 2008

Keywords:
Steroid separation
UPLC
Method validation
Cleaning validation
High speed separation

ABSTRACT

An ultra performance liquid chromatographic (UPLC) method was developed for simultaneous determination of seven steroid (dienogest, finasteride, gestodene, levonorgestrel, estradiol, ethinylestradiol, and norethisterone acetate) active pharmaceutical ingredient (API) residues. A new, generic method is presented, with which it is possible to verify the cleaning process of a steroid producing equipment line used for the production of various pharmaceuticals. The UPLC method was validated using an UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) and acetonitrile–water (48:52, v/v) as mobile phase at a flow rate of 0.55 ml/min. Method development and method validation for cleaning control analysis are described. The rapid UPLC method is suitable for cleaning control assays within good manufacturing practices (GMP) of the pharmaceutical industry.

1. Introduction

In pharmaceutical industry the cleaning procedure is one of the most important tasks to avoid the cross contamination for subsequent batches manufactured in the same equipment. Analytical methods used to determine residuals or contaminants should be specific for the substance or the class of substances to be assayed (e.g., API residue, detergent residue) and be validated prior to cleaning validation [1–3].

Guidelines recommend thin layer chromatography (TLC), UV-photometric, total organic carbon analysis (TOC), conductivity, gas chromatography (GC) and conventional high performance liquid chromatography (HPLC) methods for cleaning control or validation [4].

The use of other analytical methods, including capillary gas chromatography [5], over-pressured layer chromatography (OPLC) [6] or micellar electrokinetic chromatography (MEKC) [7], have also been described. Ion mobility spectrometry (IMS) [8] and TOC [9] have the advantage of speed over the above-mentioned methods but TOC is not specific and IMS is usually not available at pharmaceutical manufacturing facilities. Liquid chromatography–mass spectrometry (LC–MS) [10,11] and ultra performance liquid chromatography–mass spectrometry (UPLC–MS) [12] techniques applied in pharmaceutical cleaning verification have the advantage of improved sensitivity, selectivity and general applicability even for UV-inactive compounds. However, these techniques are more expensive than the other techniques mentioned above and not widespread yet in cleaning control analysis. Nowadays HPLC–UV is the most commonly applied technique for cleaning control and validation [13–18].

In liquid chromatography, the analysis time can be reduced by using small columns packed with sub-2 μm particles. In addition, with sub-2 μm particles, due to the higher efficiency and smaller retention volume, sensitivity is also improved, compared to conventional HPLC. However, extra column effects are more significant for scaled down separations, therefore it is essential to minimize extra column dispersion. A dedicated low dispersion system for ultra-high pressure separation (UPLC) with the particle size of stationary phases reduced down to 1.7 μm, small dwell and extra column volume is able to work up to 1000 bar (15,000 psi). In such a way the analysis time could be reduced down to 1–3 min, without the loss of resolution and sensitivity [19,20]. It seems that in the future UPLC systems with elevated pressure and/or temperature will replace the conventional HPLC gradually in all areas of liquid chromatography including pharmaceutical analysis [21].

The aim of this study was to demonstrate the applicability of UPLC to these purposes by developing, validating and applying an UPLC/UV method to determine the residues of UV-active steroid hormones such as dienogest, finasteride, gestodene, levonorgestrel, estradiol, ethinylestradiol, and norethisterone acetate in support of cleaning control and validation for seven different pharmaceutical

* Corresponding author.
E-mail address: fekete.szabolcs1@chello.hu (S. Fekete).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.12.027

formulations of a pilot producing line. Some of these formulations contain more steroid actives in different combinations.

A variety of chromatographic methods are described in the literature for the separation and determination of the seven steroid active pharmaceutical ingredients listed above. For a review see Ref. [22]. However, no paper can be found in the literature in which the simultaneous determination of these steroids are described and applied for cleaning control analysis.

2. Experimental

2.1. Reagents and solvents

Acetonitrile, methanol and ethanol (gradient grade) were purchased from Merck (Darmstadt, Germany). Water was prepared freshly using a Milli-Q® equipment (Milli-Q gradient A10 by Millipore).

The reference materials and samples were produced by Gedeon Richter Plc (Budapest, Hungary). Their purity was as follows: dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9(10)-diene-3-one) 99.84%, finasteride (N-tert-butyl-3-oxo-4-aza-5α-androst-1-ene-17(-carboxamide) 99.86%, gestodene (13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,15-dien-20-yn-3-one) 99.72%, levonorgestrel (13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (−)) 99.94%, estradiol (estra-1,3,5(10)-triene-3,17-β-diol) 98.34%, ethinylestradiol (19-nor-17α-pregn-1,3,5(10)-trien-20-yn-3,17-diol) 99.90% and norethisterone acetate (17-acetoxy-19-nor-17-pregn-4-en-20-yn-3-one) 99.78%.

Waters UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was purchased from Waters Ltd., Budapest.

Swabs (sterile gauze sheet 6 cm × 6 cm) for sampling were purchased from Nagév Kft Kötszer Divizió (Budapest, Hungary).

2.2. Equipment

Throughout the measurements a Waters Acquity UPLC™ system with Empower software from Waters Ltd., Budapest, Hungary, was employed. Solvent optimization was performed using Dry Lab 2000 Plus chromatography optimization software (Molnar-Institute Berlin, Germany).

2.3. Chromatographic conditions

The mobile phases were prepared by mixing appropriate amount of HPLC gradient grade acetonitrile and Milli-Q water. The mixtures were degassed by sonication for 5 min.

The stock solutions of reference standards (dienogest, finasterid, gestodene, levonorgestrel, estradiol, ethinylestradiol, and norethisterone acetate) were dissolved in methanol (1000 μg/ml). The solutions for method development were diluted from the stock solutions with 45% water + 55% acetonitrile solvent mix. The concentration of the test analytes was equivalent with the calculated maximum allowable residue limit in the reference solution. For basic gradient runs and mobile phase optimization, swabbed and rinsed blank solutions were also injected. Blank solutions were sampled from stainless steel, plexi-glass, polytetrafluorethylene (PTFE), silicone and textile model surfaces. These surfaces represented the sampling points of the product line.

Two gradients—two column temperature basic model runs were carried out and DryLab software was used to predict the optimal solvent ratio, which would give sufficient resolution (Rs > 1.5) between the compounds and peaks originated from sampling matrix. A Waters UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was used for basic runs and optimization. Linear gradients with 6 and 18 min at 45 and 65 °C column temperature at a flow rate of 0.4 ml/min were used. Detection at 210 nm was applied. Gradient program, column temperature, injection volume and flow rate were optimized.

2.4. Sample preparation

Sterile gauze sheets for swabbing were soaked in water and sonicated for 10 min. After decantation the ultrasonic wash was repeated in water and then in methanol. After the last washing, the gauze sheets were dried on sintered glass filter (GI pore size) under vacuum. After total drying, the gauze sheets were stored in screw-capped bottle until usage. Before sampling and model swabbing, tampons were made from the gauze sheets in the following way: the sheets were folded into half diagonally and piled in a dish. Wipes were soaked with methanol before sampling. In the course of sampling triangularly folded gauze tampon was handled with non-corrodible metal forceps. Sampled surfaces were swabbed from top to bottom, the wipe folded and the surface swabbed from left to right. The wipes were placed in test tubes and 10 ml of freshly prepared sample solvent (acetonitrile–water 45:55, v/v) was added then sonicated for 5 min to produce the complete dissolution of compounds from the wipe. Finally, each extracted sample solution was poured into a centrifuge tube and was centrifuged for 5 min at 4000 rpm.

Rinse-sampling was performed with 60% ethanol. The volume of the rinsing liquid for sampling points was calculated in accordance with the area of rinsed surface and with analytical limit.

Several types of swabs (foam, polyester, cotton and gauze) were tested. The commercially available prefabricated swabs are expensive and no advantage to sterile gauze sheets was observed. The gauze sheet requires a simple and well standardizable pre-treating prior to application.

Recovery studies were performed with different extracting solvents. Methanol–water and acetonitrile–water solvent mixtures in different composition were tested. The stronger the solvent was the higher the obtained recovery was. To avoid the peak broadening originating from sample solvent, acetonitrile–water 45:55 (v/v) solvent mix was considered as a suitable extracting solvent for swab samples. In case of rinse-sampling 60% ethanol provided an adequate recovery without chromatographic peak distorting.

2.5. Establishing cleaning limits

The acceptable limit for the drug residue must ensure the absence of cross contamination for subsequent batches manufactured in the affected equipment [23]. FDA's guidance for determining residue limits requires a logical, practical, achievable and verifiable determination practice [2].

The hormone pilot plant of Gedeon Richter Plc is used for technology development. Until a technology and the equipment line are not finalized, the cleaning process cannot be validated, but just verified. It has to be taken into consideration that the product list and technologies may get modified. In the pilot hormone plant the limits are calculated from "A" product to any subsequent product. This concept allows the establishment of limits not depending on the order of production. Preset limits for the analytical procedures can be applied. It also facilitates the pilot plant to produce any subsequent product in the equipment line.

The basic principle of cleaning verification/validation is that the patient should not take more than 0.1% of the standard therapeutic dose (effective dose). The calculation formula is based on the dosage criteria [24,25].

$$\mathrm{MAC} = \frac{\mathrm{STD}}{\mathrm{SF}} \cdot \left(\frac{\mathrm{SBS}}{\mathrm{LWSD}}\right)_{\mathrm{min}}$$

MAC is the maximum allowable carryover, STD is the minimal daily dose (active weight) of previous product, SF is a safety factor (1000),

SBS is the smallest batch size of the subsequent product and LWSD is the maximum daily dose (product weight) of the following product.

An additional criterion is the 10 ppm (part per million) limit [25]. According to the 10 ppm criterion not more than 10 ppm of the previously manufactured product is allowed to appear in the subsequent product. If the value, which is obtained from the calculation based on the dosage criterion, is greater than 10 ppm, then the 10 ppm criterion is applied.

The acceptance limit for residues (LA) is expressed in μg/dm^2.

$$\mathrm{LA} = \frac{\mathrm{MAC} \cdot A \cdot R}{\mathrm{TA}}$$

LA is the acceptance limit, A is the sampling area, R is the recovery of the sampling method and TA is the total production line area.

2.6. Method validation

The method validation was performed in accordance with the recent guidelines [26–28].

2.6.1. Specificity

The surface of the equipment line consists of mostly (>95%) stainless steel but there are critical surfaces, which are made of plexi-glass, polytetrafluorethylene, silicone and textile. These specific surfaces are hard to clean so it is necessary to sample these areas during the cleaning verification/validation process. During the specificity study all types of the sampling surfaces were investigated.

To prove that the determination of active residues is selective and free from any disturbing effects, reference solutions, blank and spiked solutions sampled from stainless steel, plexi-glass, PTFE, silicone and textile model surfaces and placebo solutions were injected. Resolution of Rs > 1.5 was achieved between the actives, matrix and placebo peaks, therefore the method can be considered as a specific method for these seven compounds.

2.6.2. Linearity of response

For each compound the linearity of response was assessed by injecting standards prepared in sample solvent. The concentration range of compounds was investigated from the quantitation limit (QL) up to the 150% of the maximum theoretical value (range: 0.12–4.55 μg/ml for dienogest, 0.05–8.00 μg/ml for estradiol, 0.05–1.51 μg/ml for ethinylestradiol, 0.10–2.03 μg/ml for finasterid, 0.15–1.04 μg/ml for gestodene, 0.30–1.00 μg/ml for levonorgestrel and 0.35–8.00 μg/ml norethisterone acetate).

The results were analyzed by linear regression. The correlation coefficients, r^2, were found $r^2 > 0.99$, confidence interval of Y intercept (P = 95%) contained the origin and the residuals plotted uniformly and randomly around the regression line in each case.

2.6.3. Accuracy

Samples for recovery test were prepared as follows: reference materials in the range of 50–150% of limit concentration (n = 6) were spiked to 10 cm × 10 cm model surfaces. All types of real sampled surfaces of equipment line were investigated.

Reference compounds were pipetted from stock solution (dissolved in methanol) and spread evenly on the model surfaces and allowed to dry. Wipes were impregnated with methanol and the dry model surfaces were wiped from top to bottom, the wipe folded and the surface wiped from left to right. The wipes were placed in test tubes and 10 ml of freshly prepared sample solvent (acetonitrile–water 45:55, v/v) was added and then sonicated for 5 min to produce the complete dissolution of compounds from the wipe. Finally, each extracted sample solution was replaced to centrifuge tube and centrifuged for 5 min at 4000 rpm.

Recovery of rinsed samples was modeled with the rinsing of a 10 cm × 10 cm textile sheet. Reference compounds were pipetted from stock solution (dissolved in methanol) to the textile sheet. After drying, 25 ml of ethanol–water 40:60 (v/v) solvent mix was used to rinse the model sheet to a 100-ml beaker. The extract was replaced into a centrifuge tube and was centrifuged for 5 min at 4000 rpm.

In all cases sample concentrations were determined by reference to a calibration line (3 points) constructed from standards containing the respective analyte in 50–150% around the analytical limit concentration.

From silicone rubber surface the recovery (in case of finasterid, gestodene, levonorgestrel and norethisterone acetate) did not exceed the value of 70%. Additional experiments were performed, in an attempt to increase the poor recovery from silicone. Wipes were impregnated with different types of organic solvents such as methanol, ethanol and acetonitrile. Both aqueous/organic solvent mixes as well as swabbing with second wipe were also tried but these experiments proved to be unnecessary. It did not improve the recovery significantly. It is recommended to apply these silicone surfaces (gaskets) as dedicated parts of the equipment.

2.6.4. Precision

Precision was examined by the relative standard deviation (R.S.D.) of recovery data (in 6 concentration points for each compound on different surfaces). Intermediate precision was examined by repeated recovery test by two operators. The applied criterion in our laboratory for precision, that R.S.D. of recovery results (n = 6)

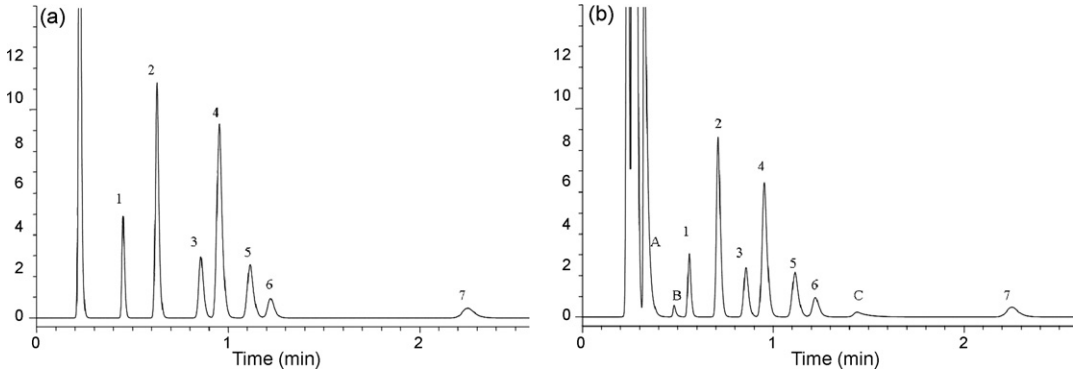

Fig. 1. Chromatogram of reference solution (a) and spiked sample swabbed from silicone surface (b). Chromatographic conditions: waters UPLC™ BEH C18 1.7 μm (50 mm × 2.1 mm) column, mobile phase: acetonitrile–water (48:52, v/v), flow rate: 0.55 ml/min, column temperature: 50 °C, injection volume: 5 μl, detection: 210 nm. Compounds: (1) dienogest (0.46 μg/ml), (2) estradiol (0.46 μg/ml), (3) ethinylestradiol (0.14 μg/ml), (4) finasterid (1.0 μg/ml), (5) gestodene (0.48 μg/ml), (6) levonorgestrel (0.48 μg/ml) and (7) norethisterone acetate (0.46 μg/ml). A, B and C peaks of matrix origin.

Table 1
Validation data.

Parameter	Dienogest	Estradiol	Ethinylestradiol	Finasterid	Gestodene	Levonorgestrel	Norethisterone acetate
Specificity[a]	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Linearity							
Correlation	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99
Intercept[b]	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Residuals[c]	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Accuracy[d]							
Steel	91.0%	81.7%	91.5%	81.2%	72.0%	73.2%	84.4%
Plexi-glass	75.3%	78.2%	70.3%	74.2%	71.5%	70.1%	73.1%
PTFE	85.0%	88.4%	85.6%	78.1%	84.5%	82.1%	87.3%
Textile	99.0%	97.5%	96.1%	92.5%	82.4%	85.2%	97.9%
Silicone	87.4%	77.4%	73.4%	60.5%	57.1%	55.2%	40.9%
Precision[e]							
Steel	4.8%	5.8%	5.1%	9.6%	12.8%	8.4%	5.8%
Plexi-glass	9.4%	6.8%	10.9%	10.6%	7.7%	8.7%	9.3%
PTFE	5.8%	9.2%	3.5%	7.4%	6.2%	9.2%	9.1%
Textile	8.5%	3.7%	10.1%	10.9%	10.1%	9.7%	3.2%
Silicone	5.4%	12.8%	5.2%	8.9%	8.2%	14.9%	21.1%
Limit of quantitation[f]	0.12 μg/ml 1.2 μg/wipe	0.05 μg/ml 0.5 μg/wipe	0.05 μg/ml 0.5 μg/wipe	0.10 μg/ml 1.0 μg/wipe	0.15 μg/ml 1.5 μg/wipe	0.30 μg/ml 3.0 μg/wipe	0.35 μg/ml 3.5 μg/wipe
Limit of detection[g]	0.06 μg/ml 0.6 μg/wipe	0.02 μg/ml 0.2 μg/wipe	0.02 μg/ml 0.2 μg/wipe	0.05 μg/ml 0.5 μg/wipe	0.07 μg/ml 0.7 μg/wipe	0.15 μg/ml 1.5 μg/wipe	0.20 μg/ml 2.0 μg/wipe

[a] To prove specificity, reference solutions, blank and spiked solutions sampled from stainless steel, plexi-glass, PTFE, silicone and textile model surfaces and placebo solutions were injected. The criterion for resolution was Rs > 1.5 between any actives, matrix and placebo peaks.
[b] Confidence interval of *Y* intercept (*P* = 95%) should contain the origin.
[c] Residuals should spread uniformly and randomly around the regression line.
[d] Mean value of the recovery in the range of 50–150% of limit concentration (*n* = 6).
[e] Relative standard deviation (R.S.D.) of recovery data.
[f] Concentration, where R.S.D.% of repeated peak areas (*n* = 5) not exceed 10%.
[g] Concentration, where R.S.D.% of repeated peak areas (*n* = 5) not exceed 30%.

should be lower than 15% [4]. With the exception of silicone surface the R.S.D. < 15% criterion was successfully accomplished.

2.6.5. Limit of quantitation and detection

Quantitation limits (LOQ) and detection limits (LOD) were determined by the R.S.D. of five repeated injections of standard solutions. In the laboratory R.S.D. < 10% for LOQ concentration and R.S.D. < 30% for LOD concentration criteria are used for the methods of cleaning verification. Another term for LOD – in the laboratory – is that it must be lower than the 50% of analytical limit. The sensitivity of the method is proved to be sufficient for each compound.

2.6.6. Stability of sample and stock solutions

The stability of sample solution was studied on standard as well as on test-sample (spiked and swabbed from stainless steel) solutions at the concentration of cleaning limits (0.46 μg/ml dienogest, 0.46 μg/ml estradiol, 0.14 μg/ml ethinylestradiol, 1.0 μg/ml finasterid, 0.48 μg/ml gestodene, 0.48 μg/ml levonorgestrel and 0.46 μg/ml norethisterone acetate). The solutions were stored in a sample compartment and are chromatographed 12 times within a 24-h period. R.S.D. of peak areas was calculated, and peak areas were plotted against the time. R.S.D. < 2% criterion is used for the methods of cleaning verification. Peak areas should spread uniformly and without tendency when plotted against the time for each compound. The standard and test solutions were proved to be stable for each compound within a 24-h period. There were no detectable degradants on the chromatograms.

For the establishment of the stability of standard stock solution, 3 standard stock solutions were prepared and stored at refrigerator for one week. For the measurements freshly diluted solutions were used and injected every day, and the differences from the peak areas injected at the beginning were calculated. No more than 2% differ-

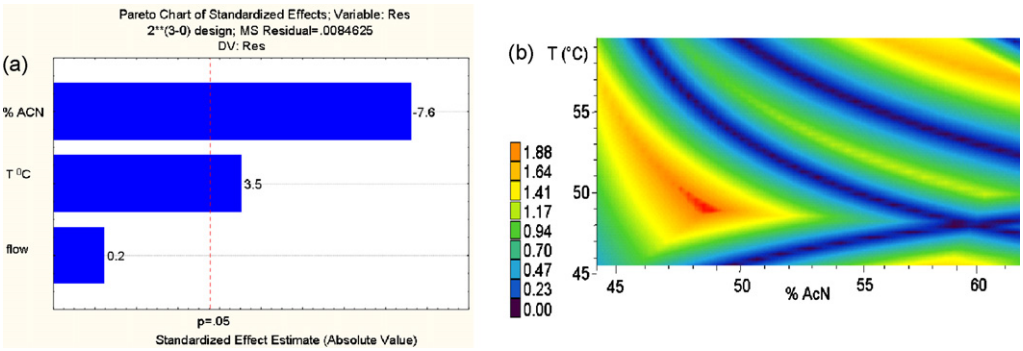

Fig. 2. Pareto chart (a) and resolution map (b) of robustness test. (a) Displays the standardized effects of column temperature, acetonitrile % of mobile phase and flow rate on critical peak resolution (*P* = 0.05) and (b) resolution map shows that the operating column temperature (50 °C) and acetonitrile content of mobile phase (48%) provides the highest peak resolution.

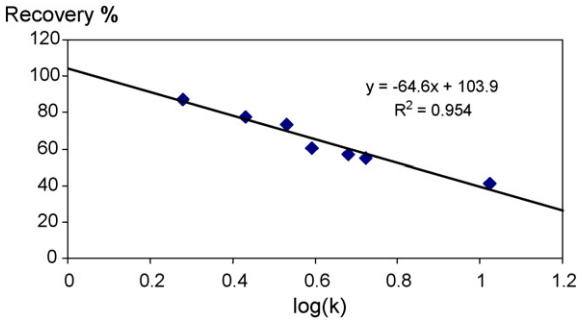

Fig. 3. Fitted linear curve to the recovery data in the function of log (k) values.

ence was observed each day. The stock solutions of the reference substances were considered as stable, for at least 7 days.

2.6.7. Robustness

In order to test the robustness of the UPLC method, a 2^3 standard experimental design was applied to estimate the effect of oven temperature, mobile phase content and flow rate on critical resolution.

The temperature was adjusted to 48 and 52 °C, the mobile phase acetonitrile content was varied to 47 and 49% and the flow rate of 0.54 and 0.56 ml/min were applied in accordance with the experimental design. The effects were displayed on Pareto chart.

Computer-facilitated HPLC method development using a simulation program can be useful for the investigation of the influence of the respective chromatographic parameters on the separation and consequently on the robustness of a given RP-HPLC method [29]. The effects of chromatographic parameters were predicted via the resolution map, based on the data generated during the basic model runs of method development.

3. Results and discussion

The purpose of this study was to develop a fast method for the cleaning validation process of the pilot equipment line. A fast, isocratic UPLC method has been developed to separate the seven steroids and matrix compounds with baseline resolution within 2.5 min (Fig. 1), and can be applied for the cleaning control analysis of the hormone pilot equipment line. A systematic computer assisted method development was applied to find the optimal separation conditions.

3.1. Method validation

The UPLC method was validated. Data are summarized in Table 1.

For system precision and suitability, five repetitions of injection from standard solution were used. The acceptance criteria for system suitability were as follows: retention factor (k) of dienogest is greater than 1.0, the resolution (Rs) of gestodene and levonorgestrel peak pair is at least 1.5, and the R.S.D. of peak areas generated by five injections is lower than 2.0% for each compound. Specificity, linearity over the range of interest, accuracy (recovery from different types of surfaces) in the range of 50–150% of analytical cleaning limit, precision and limit of quantitation and detection were determined.

The mean accuracy (recovery) from stainless steel, plexi-glass, PTFE and textile are acceptable for this type of analysis (recovery >70%), they are corrected by a recovery factor during routine analysis. From silicone rubber surface the recovery (in case of finasterid, gestodene, levonorgestrel and norethisterone acetate) did not exceed the value of 70%. The more apolar the compound to be removed from apolar silicone model surface, the worse its recovery.

For the robustness test a 2^3 standard experimental design was applied to estimate the effect of chromatographic parameters. The Pareto chart (obtained by Statistica 8.0 software) of experimental design shows that acetonitrile content of mobile phase and column temperature take significant effect on resolution (Fig. 2a). Resolution map generated by the simulation software indicate that working condition of the method is at the optimum point (Fig. 2b).

3.2. Recovery study

The surface of equipment is mostly made of stainless steel but there are some gaskets made of silicone rubber. These silicone parts are the most critical in correlation with cleaning and sampling in our experience. The silicone surfaces proved to be more critical when steroids are produced than in the case when basic or acidic compounds are manufactured in the equipment. Recovery data from silicone are poor when steroids are swabbed compared to any other types of API. When recovery is lower than 50%, the critical parts became dedicated to the production of the formula containing the problematic API.

A good correlation was found between recovery and the retention factor (k) measured in reversed phase isocratic system (Fig. 3).

4. Conclusion

On the basis of this study, it appears that the use of UPLC for the quantification of API residues in cleaning validation samples in product formulation area is practical. The time reducing and solvent saving characteristics of UPLC method are very advantageous, compared to the most widely used conventional HPLC technique. The enhanced sensitivity of the UPLC–UV method compared to conventional HPLC does not necessitate the use of a mass spectrometry detector, which is expensive and not widespread in cleaning control analysis. The concept of applying a generic method for several API residues for a product line is feasible and practical if the structure and properties of compounds to be determined are similar.

Recovery results of analytical method validation show, that silicone rubber surfaces are critical during the cleaning and the sampling process. It is recommended to apply these silicone gaskets as dedicated parts of the equipment.

Acknowledgement

The authors wish to acknowledge Mária Langer and the staff at the Formulation Development Laboratory for their support.

References

[1] FDA, Guide to Inspections of Validation of Cleaning Processes, 1993.
[2] Guide to Inspections of Validation of Cleaning Processes, Reference Material for FDA Investigators and Personnel, Food and Drug Administration, Washington, DC, July, 1993, pp. 1–6.
[3] Cleaning Validation Guidelines—Good Manufacturing Practice, Health Products and Food Branch Inspectorate, Canada, 2004.
[4] Active Pharmaceutical Ingredient Committee, Guidance on Aspects of Cleaning Validation in Active Pharmaceutical Ingredient Plants, 2000.
[5] T. Mirza, R.C. George, J.R. Bodenmiller, S.A. Belanich, J. Pharm. Biomed. Anal. 16 (1998) 939–950.
[6] Z. Katona, L. Vincze, Z. Végh, Á. Trompler, K. Ferenczi-Fodor, J. Pharm. Biomed. Anal. 22 (2000) 349–353.
[7] M.B. Boca, E. Pretorius, C. Kgaje, Z. Apostolides, J. Pharm. Biomed. Anal. 46 (2008) 631–638.
[8] R. Debono, S. Stefanou, M. Davis, G. Walis, Pharm. Technol. 26 (2002) 72–78.
[9] K.M. Jenkins, A.J. Vanderwielen, J.A. Armstrong, L.M. Leonard, G.P. Murphy, N.A. Piros, J. Pharm. Sci. Technol. 50 (1996) 6–15.
[10] E.L. Simmonds, W.J. Lough, M.R. Gray, J. Pharm. Biomed. Anal. 40 (2006) 631–638.
[11] L. Liu, B.W. Pack, J. Pharm. Biomed. Anal. 43 (2007) 1206–1212.
[12] K.J. Fountain, M. Van Wingerden, D.M. Diehl, LC–GC North-Am. 25 (2007) 66–67.
[13] J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, J. Pharm. Biomed. Anal. 23 (2000) 421–428.

[14] M.J. Nozal, J.L. Bernal, L. Toribio, M.T. Martin, F.J. Diez, J. Pharm. Biomed. Anal. 30 (2002) 285–291.
[15] T. Mirza, M.J. Lunn, F.J. Keeley, R.C. George, J.R. Bodenmiller, J. Pharm. Biomed. Anal. 19 (1999) 747–756.
[16] M.B. Boca, Z. Apostodiles, E. Pretorius, J. Pharm. Biomed. Anal. 37 (2005) 461–468.
[17] T.T. Fazio, A.K. Singh, E.R.M. Keodor Hackmann, M.I.R.M. Santoro, J. Pharm. Biomed. Anal. 43 (2007) 1495–1498.
[18] R. Klinkenberg, B. Streel, A. Ceccato, J. Pharm. Biomed. Anal. 32 (2003) 345–352.
[19] M.E. Swartz, B. Murphy, Am. Lab. 37 (2005) 22–35.
[20] M.E. Swartz, J. Liq. Chromatogr. 28 (2005) 1253–1263.
[21] S. Görög, Trends Anal. Chem. 26 (2007) 12–17.
[22] S. Görög, Anal. Sci. 20 (2004) 767–782.
[23] R.J. Forsyth, D.V. Haynes, Pharm. Technol. 22 (1998) 104–112.
[24] D.A. LeBlanc, Pharm. Technol. 22 (1998) 136–148.
[25] G.L. Fourman, M.V. Mullen, Pharm. Technol. 17 (1993) 54–60.
[26] ICH Harmonised Tripartite Guideline, Q2A, Text on Validation of Analytical Procedures, October 27, 1994.
[27] ICH Harmonised Tripartite Guideline, Impurities in New Drug Products, Q3B(R2), Current Step 4 version, 2 June 2006.
[28] G.A. Shabir, J. Chromatogr. A 987 (2003) 57–66.
[29] I. Molnár, J. Chromatogr. A 965 (2002) 175–194.