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Cleaning validation procedure eased by using overpressured layer chromatography

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Abstract

In the manufacturing plants of many pharmaceutical companies the reaction apparatus is suitable to produce different active pharmaceutical ingredients. After completing the production of a compound the equipment should be cleaned in order to avoid the cross contamination in the next lot of the other products. In the authors' laboratory several chromatographic methods were introduced to measure the amount of the residual substances remaining on the surface of the apparatus after the cleaning procedure. A sensitive and fairly rapid overpressured layer chromatographic (OPLC) procedure — suitable to separate and control five steroid hormone compounds (allylestrenol, estradiol, ethynodiol diacetate, levonorgestel, norethisterone) produced in the same equipment at different times — was developed and validated.

Keywords: Cleaning validation; Overpressured layer chromatography (OPLC); Analytical method validation; Allylestrenol; Estradiol; Ethynodiol diacetate; Levonorgestel; Norethisterone

1. Introduction

The severe regulations and requirements depicted in current good manufacturing practice (CGMP) [1] on equipment cleaning demand the written proof of the cleanliness of the apparatus used for manufacturing of active pharmaceutical ingredients.

In the end of the cleaning procedure the effectiveness of the cleaning is checked using a validated analytical method suitable to investigate the traces of residues. The validation and the documentation of the equipment cleaning are to certify that the amount of the cross contaminants in the next prepared batch can be reduced below a predetermined limit using the cleaning method. The limit — the maximum allowable carryover of the compound — can be calculated with knowledge of the whole surface of the equipment (100 m^2) and the smallest batch size made in the same reaction apparatus. Three grams (0.01%) of the previous manufactured steroid is allowed to remain in the equipment taking into account a

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30-kg batch size. This assumption was supported by calculating the allowable non-toxic carryover value based upon the toxicity data of the compounds using the method described by Layton and co-workers [2].

During the cleaning validation methanol dabbled swab samples were taken from 30 hardest-to-clean areas of the apparatus used. On a swabbed surface (100 cm^2) maximum 300 μg of residual substances should be detected, which corresponds to a 30-kg batch size and 100-m^2 surface. A validated, sensitive and selective analytical method should be used for the monitoring of the effectiveness of equipment cleaning in cleaning validation. In the authors' laboratory a new, overpressured layer chromatographic procedure was developed. It is suitable to separate and control five steroid hormone compounds shown in Fig. 1 produced in the same equipment at different times.

Overpressured layer chromatography (OPLC) is a planar layer liquid chromatographic method [3]. The vapor phase of the eluent is completely eliminated by keeping the stationary phase under pressure during the development. The eluent is delivered through the sorbent layer by forced flow with constant and optimal flow rate during the development, using a pump system. The main advantages of this method are the highly effective separation, the short analysis time and a low eluent-consumption.

The number of applied samples can be multiplied and can be analyzed simultaneously using a two-directional development, the special possibility of the overpressured layer chromatographic method [3]. The analysis time can be decreased further by this development mode, which is very advantageous in an in-process laboratory.

The mobile phase of the procedure was optimized by PRISMA model according to Nyiredy et al. [4]. PRISMA is a three-dimensional model correlating Snyder's solvent classification and the proportion of the eluent constituents. Using this optimization method we have chosen solvents from ten different ones (apolar as well as polar solvents) providing the best separation of the five investigated steroids.

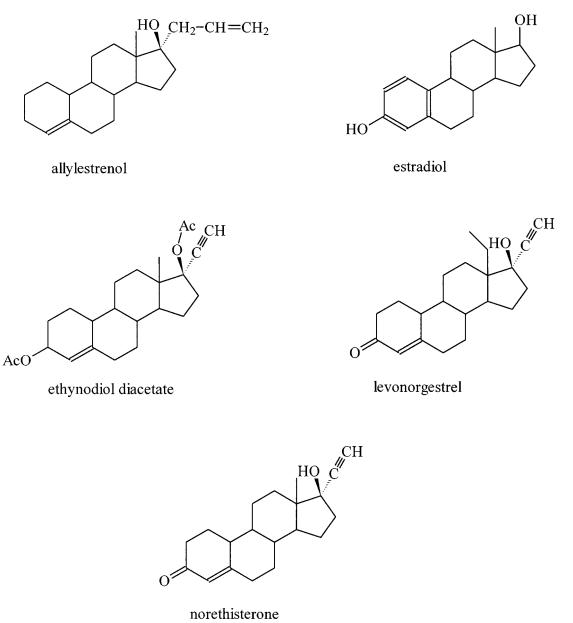

Fig. 1. Structures of manufactured and investigated active pharmaceutical ingredients.

2. Experimental

2.1. Materials

The five investigated compounds were prepared in Gedeon Richter Ltd. HPLC grade solvents were purchased from Merck (Darmstadt, Germany).

2.2. Chromatographic methods

For the optimization of the overpressured layer chromatographic method, thin-layer chromatography was used. The separations were performed on silica gel sorbent layers (Merck # 1.5554).

The overpressured layer chromatographic separations were performed by using a Personal OPLC BS 50 instrument (OPLC-NIT Engineering Ltd. Budapest, Hungary) with fine particle silica gel (Merck # 1.5548) sealed sorbent layers. Five μl of the sample solutions were applied and a two-directional development was used. Conditions during the development were the following: mobile phase: diethyl ether-*n*-hexane (6:4 (v/v));

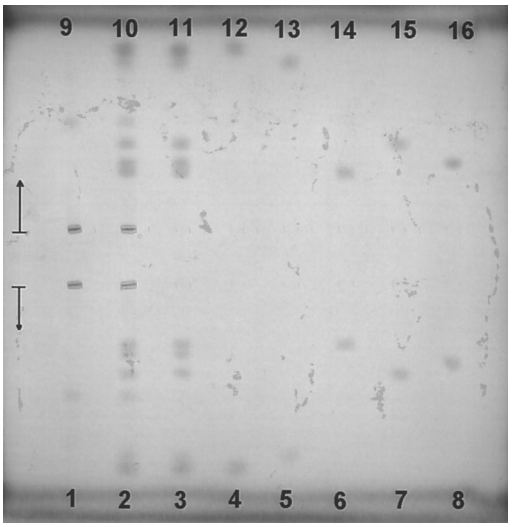

Fig. 2. Proving the specificity of the method. Application: blank sample (1, 9); 0.5 μg of the investigated substances spiked with blank (2, 10); 0.5 μg of the substances investigated (3, 11); 0.5 μg of allylestrenol (4, 12); 0.5 μg of ethynodiol diacetate (5, 13); 0.5 μg of norethisterone (6, 14); 0.5 μg of levonorgestel (7, 15); 0.5 μg of estradiol (8, 16). Development conditions: see Section 2.2. The arrows show the directions of development.

Table 1
R_f values of investigated compounds and the impurities

Compound	R_f
Allylestrenol	0.77
Ethynodiol diacetate	0.72
Impurity extracted from the swab (blank)	0.5
Levonorgestel	0.42
Estradiol	0.35
Norethisterone	0.31
Impurity extracted from the swab (blank)	0

external pressure: 5 MPa; eluent flow rate: 800 μl/min; starting rapid eluent volume: 600 μl; developing eluent volume: 7000 μl; time of development: 15 min.

The chromatograms were visually evaluated in visible light after staining (sprayed with 10% ethanolic solution of phosphomolybdic acid, then heated at 105°C for 5 min). The documentation was carried out by means of the Camag Video-Store 2 system equipped with the Hitachi HV-C20 camera (CAMAG, Muttenz, Switzerland).

2.3. Standardized sample pretreatment

1. 10×10 cm surfaces on the 30 hardest-to-clean areas of the equipment were wiped with swabs (tampon o.b., Johnson & Johnson, Budapest, Hungary) dabbled in 3 ml of methanol. The swabs were put into test tubes and stoppered afterwards.
2. The tubes were filled with 10 ml of methanol and sonicated for 20 min.
3. The swabs were removed and the obtained solutions were evaporated to dryness in vacuo at 30°C.
4. The residue was dissolved in 0.5 ml of methanol.
5. Five microlitres of the solution were applied onto the chromatoplate.

3. Results and discussion: validation of the analytical procedure

The optimized testing method is a planar-chromatographic limit test based on visual estimation. It should be decided whether the amount of contaminants are below or above the acceptable non-toxic limit. According to the guideline (Validation of Analytical Methods: Definition and Terminology) issued by International Conference on Harmonization (ICH) in 1995 [5] the following characteristics have been checked.

3.1. Specificity

The investigated compounds were applied onto the same chromatoplate separately and also from a common solution. A blank sample, the solvent extracted from the pure swab, was also chromatographed to control the place of the disturbing blank spots. A common application of the blank sample and the standard solutions was also performed.

As can be seen in Fig. 2, the separation of the investigated substances and the blank spots from each other is satisfactory.

The R_f values are summarized in Table 1.

3.2. Detection limit (DL)

Decreasing quantities of the substances were applied onto the same chromatoplate. The applications (the common solutions of substances of different concentration) were in equal volume. DL was determined as the smallest visible quantity.

The detection limit is 0.03 μg for allylestrenol, 0.05 μg for estradiol, ethynodiol diacetate, levonorgestel and norethisterone, respectively. The allowed carryover for these active pharmaceutical ingredients is 300 μg from a 100 cm^2 surface, in this case 0.75 μg can be seen on the chromatographic plate (calculated from the volume of application and the recovery). Every detection limit is less than 10% of the allowed carryover (0.75 μg), therefore the sensitivity of evaluation is suitable for this cleaning validation task.

Taking into consideration the purpose and the special characteristics of the investigated substances and the method, further measurements are required.

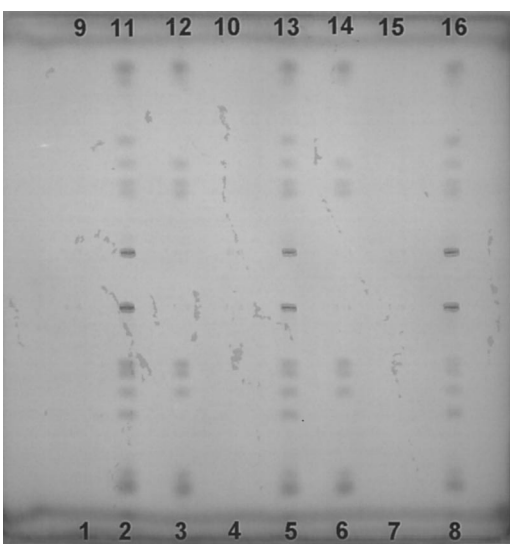

Fig. 3. Investigation on recovery. Application: the material remained on the surfaces after cleaning (1, 4, 7, 9, 10, 15); swabbing from three surfaces (100 cm^2) (2, 5, 8, 11, 13, 16); reference solution: 0.5 μg of each investigated compounds (3, 6, 12, 14). Development conditions: see Section 2.2.

3.3. Determination of recovery of the compounds from the swabbed surface

The recovery is the amount of the substance swabbed from the cleaned surface. A known quantity, 100 μg of allylestrenol, ethynodiol diacetate, estradiol, levonorgestel and norethisterone in solution were introduced to a 100-cm^2-glass surface. After that the surface was dried and cleaned by a swab. The swabs were extracted by methanol. The cleaned surface was swabbed again and the swab was extracted. The amount of the substances remained on the surface was determined after extracting by methanol, too. The steroid content of the extract was controlled in triplicate by semi-quantitative OPLC method according to Section 2.2.

The recovery was higher than 50% as presented in Fig. 3, therefore it can be accepted.

3.4. Investigation on stability of analyte in the solution and on the chromatographic plate

It should be verified that the sample in the solution and on the plate before and during the chromatographic development is stable for the time of pretreatment.

The investigated compounds were dissolved in methanol 0, 15, 30, 45 and 60 min before the application. Two micrograms of the substances were applied at the same time (in the 60th min) in order to investigate the stability of analytes in solution.

The stability of analytes on the chromatoplate before development was determined by dissolving and applying the compounds at a time of 0, 15, 30, 45, 60 min the solutions corresponding to 2 μg of substances on the same chromatoplate side by side and chromatographing immediately.

Allylestrenol, norethisterone, levonorgestel and estradiol are stable for 60 min both in the solution and on the chromatographic plate before development, ethynodiol diacetate is stable for 60 min in the solution and for 15 min on the chromatographic plate.

The stability of analytes during chromatographic development was investigated by a two-dimensional and two-directional development,

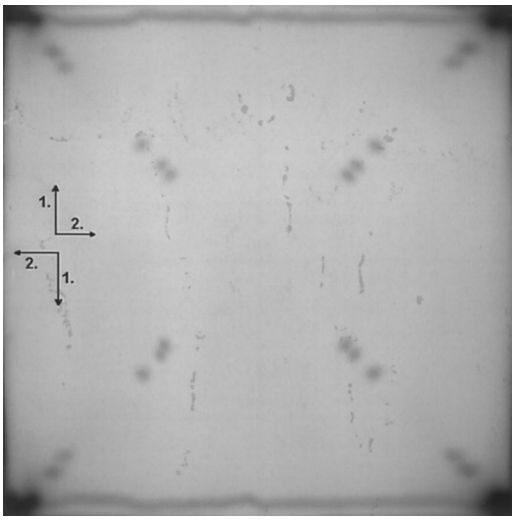

Fig. 4. Testing the stability of the analytes during chromatographic development by a two-dimensional, two-directional separation. Development conditions: see Section 2.2. The arrows show the directions of development.

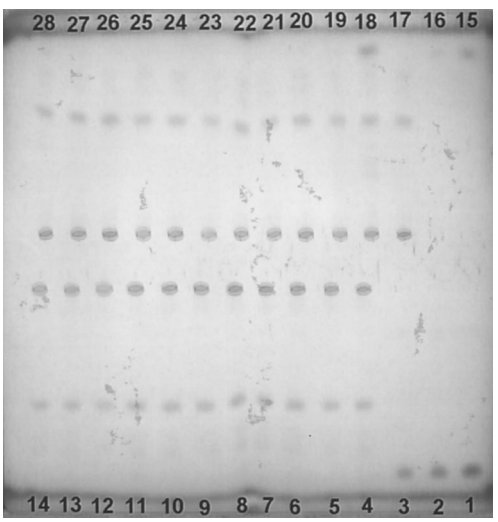

Fig. 5. Proving the cleanness of the equipment by overpressured layer chromatographic method after manufacturing of allylestrenol (application in practice). Application: 0.3 μg (1), 0.15 μg (2), 0.1 μg (3), 0.05 μg (15), 0.03 μg (16) of allylestrenol (reference); swab samples from hardest-to-clean areas of the equipment (4–14, 17–28). Development conditions: see Section 2.2.

using the system eluent in both directions. As can be seen in Fig. 4, the spots are situated in the diagonal of the chromatogram, therefore degradation during the development did not occur.

3.5. Application in practice: equipment cleaning after the manufacturing of allylestrenol

The result of equipment cleaning after production of allylestrenol active pharmaceutical ingredient is presented in Fig. 5. As can be seen, the amount of the substance remained on the area No. 18 of the equipment was 0.1 μg, which is below the acceptable limit (0.75 μg). All other results are below the detection limit.

4. Conclusions

The OPLC-based system to control the efficiency of the equipment cleaning is suitable for separation of steroid hormones and the impurities extracted from the swab. It is very quick due to the possibility of the two directional-development (about 30 times faster than an HPLC method). The method is cost-effective by operating with small volume of solvents. The cost of the validation can be reduced (only one validation is necessary for five compounds). The sensitivity is approximately the same as that of the corresponding HPLC method.

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