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Development and Validation of Quantitative Determination and Sampling Methods for Acetaminophen Residues on Pharmaceutical Equipment Surfaces

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ABSTRACT. The present study concerns the development and validation of rapid and selective high performance liquid chromatographic (HPLC) method for quantitative estimation of acetaminophen residues in samples collected from pharmaceutical manufacturing equipment surfaces and the development of the methodology of establishing the allowable limits for cleaning validation. The developed swab and rinse sampling procedures and HPLC method were validated with respect to accuracy, system suitability test, specificity, linearity, limit of detection (LOD) and quantitation (LOQ). The calibration curve is linear r^2=99999 over a concentration range 0.00002–0.01 mg/ml; LOQ - 0.0002 mg/ml and LOD - 0.00002 mg/ml. *© 2018 Bull. Georg. Natl. Acad. Sci.*

Key words: acetaminophen residues, high performance liquid chromatography, cleaning validation

Cleaning validation is a critical analytical responsibility of quality system in pharmaceutical industry as Food and Drug Administration (FDA)/Good Manufacturing Practice (GMP) requirement which shows that the routine cleaning procedure effectively removes the residues from the manufacturing equipment and facilities below a predetermined level. This is necessary to ensure the quality of the next batch of different pharmaceutical products but also, to prevent cross-contamination. Residues of active pharmaceutical ingredient (API) of previous pharmaceutical product have a significant cross-contamination potential. API's residues estimation requires the development of selective and sensitive methods capable of quantitative estimation of traces remaining over the surface of manufacturing equipment after the cleaning procedure. The acceptance limit (AL) for residues on the equipment is not established in the current

regulations and should be based on logical and scientific criteria, involving the risk associated with residues of determined products [1-2].

Acetaminophen (Paracetamol) is a pain reliever and a fever reducer, used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers (CAS registry number: 103-90-2). It is white, odorless, crystalline powder, having a slightly bitter taste, freely soluble in alcohol, soluble in boiling water and in 1 N sodium hydroxide [3].

The goal of this study was to develop and validate a selective and rapid HPLC method and a swab and rinse sampling procedure for determination the residues of Acetaminophen in cleaning control samples collected from manufacturing equipment surfaces after manufacturing Ephact Flu capsules (One capsule contains 500 mg of acetaminophen, 10 mg phenylephrine hydrochloride, 2 mg chlorphenamine maleate). This active ingredient different from other APIs - phenylephrine hydrochloride and chlorphenamine maleate was selected as "the worst case" of cleaning procedure and practically insoluble in room temperature water.

Materials and Methods

Reagent and chemicals. The certified reference standard of acetaminophen was supplied by USP reference standards. The HPLC grade methanol and ethanol was purchased from Sigma-Aldrich.

Instrumentation. The HPLC grade water was prepared using Milli Q Advantage A10 purification system (Millipore, France). Polyester microswab (3×2.5×10 mm) for sampling was purchased from ITW Texwipe (USA). Cleaning procedure was performed using Microbac Forte 1 % solution as a disinfectant/detergent.

The chromatographic analysis was performed using Ag 1260 Infinity (AG Technologies, USA). The output signal was monitored and processed using Chemstation software. SONOREX™ Digital 102P Ultrasonic bath DK 102 (Germany), Analytical balance CPA 232S Sartorius (Germany) was used for sample preparation. All the measuring equipment was calibrated and qualified. Statistical assessment was performed using Microsoft Excel 2010.

Chromatographic system and conditions. The method was developed using a Luna C18(2) 250×4.6 mm, 5 μm column with an isocratic mobile phase containing a mixture of methanol and HPLC grade water (10 : 30 v/v) filtered through Durapore PVDF, 0.45 μm membrane filters and degassed; the flow rate – 1.0 ml/min; the detector wavelength - 243 nm; the injection volume – 10 μl; the column temperature was maintained at 25 °C.

Standard preparation. 0.2 mg/ml stock solution of acetaminophen in diluent – ethanol: water 1:1 v/v with. 1 ml of obtained solution was transferred to a 20 ml volumetric flask, diluted to volume with diluent and was mixed well (0.01 mg/ml).

Sample preparation (extraction procedure). Rinsing and swabbing methods, the FDA preferred techniques were used for sampling the APIs residues. The swabbing process is a subjective manual process that involves physical interaction between the swab and the even surface. The surface was wiped horizontally, starting from outside toward the center. The selected surfaces (the worst case sampling places evaluated based on risk assessment) of stainless steel of equipment ($5\times5 cm^2$) were previously cleaned with disinfectant detergent and were dried. The surface was successively wiped with one swab moistened with extraction solution – diluent (96 % ethanol). The swabs were placed in the 5 ml screw-cap test tubes containing 2 ml extraction solution. Subsequently, the tubes were placed in an ultrasonic bath for 2-3 minutes and the solutions were analyzed by HPLC.

The rinse samples from uneven surfaces (plastic brush) were collected by rinsing them with the fixed volume of the selected diluent (96 % ethanol).

Recovery rate of swab sampling from stainless steel surfaces. The selected surfaces of stainless steel ($5\times5 cm^2$) were sprayed with 100 μl of

standard stock solution and the diluent (96 % ethanol) was allowed to evaporate. Then swab sampling was performed according to swab wipe procedure as described in sample preparation. The samples were diluted with the same diluent to 2 ml.

The recovery rate, % was calculated by the formula: Rec, $\%=R_u \times 100/R_s$. Where, R_u – is the peak area of acetaminophen obtained from swab sample solution and R_s the peak area of acetaminophen obtained from standard solution.

Quantitative estimation of acetaminophen residues. The concentration (mg/ml) of acetaminophen residues was calculated by the formula: $X=R_u \times W \times D \times P/R_s \times 100$. Where, R_u is the peak area of acetaminophen obtained from the chromatogram of swab sample solution; R_s is the peak area of acetaminophen obtained from the chromatogram of the standard solution; W – mass of the weighed acetaminophen standard, mg; D – dilution factor, ml; P - purity of standard, %.

Results and Discussion

Establishing cleaning limits. The acceptable limit for the drug residues must ensure the absence of cross-contamination for subsequent batches manufactured in the affected equipment. The basic principle of cleaning validation is that the patient should not take more than 0.1 % of the standard therapeutic dose of the previous (control) product in the subsequent product which will not produce any adverse effects (dosage criteria) [1-2].

The calculation formula: $MAC=TD \times SF \times BS /LDD$, where, MAC is the maximum allowable carryover of the API residues of the previous product (mg); TD is the API minimal therapeutic dose of previous product (mg); SF is a safety factor (1/1000) for oral drug dosage form; BS is the smallest batch size of the subsequent product (mg) and LDD is the largest daily dose of the subsequent product (mg). The acceptable limit for residues in sample solution is expressed in mg/ml. For swab sample solution: $AL<MAC \times Rec \times A_s \times F/A_t \times V$ and for rinse sample solution: $AL<MAC/V$, where, AL is the acceptance limit (mg); A_s is the sampling area (cm^2); Rec is the recovery rate of the sampling method and A_t is the total production line area (cm^2); V – the volume of swab/rinse sample (ml).

The calculated AL of acetaminophen for swab sample solution is 17.984 mg/ml, for rinse sample solution –4.606 mg/ml. To estimate the determined concentration of acetaminophen residues in sample solution should not be more than the AL (acceptance criteria).

Optimization of chromatographic system conditions and sampling method. The chromatographic conditions were achieved by optimizing the chromatographic system parameters: wavelength of detection, composition of mobile phase, flow rate, nature of stationary phase and checking the system suitability parameters: theoretical plates, tailing factor (USP peak symmetry), and peak purity.

The swab sampling method was developed in order to obtain a suitable recovery. The surface (sampling area - 5×5 cm^2) was successively wiped with one micro polyester swab (3×2.5×10 mm) moistened with diluent – 96 %ethanol. The swabs were spiked with different quantities of acetaminophen. The optimal conditions were achieved with diluent -96 %ethanol (nontoxic and easy to remove from surfaces by deionized water after sampling; also, Acetaminophen is freely soluble in ethanol) and sonication time of 2-3 minutes. The average recovery rate of swab sampling from stainless steel surfaces is 95.07 %.

Method validation. The analytical method was validated with respect to system suitability test, specificity, linearity-range, limit of detection (LOD) and limit of quantitation (LOQ). The stability of standard solution of acetaminophen was studied as well. This study was performed in accordance with established ICH Q(2) guideline and United States Pharmacopoeia (USP).

Specificity. The specificity of the method was checked by injecting the standard solution, the

spiked swab sample solution and the negative control (placebo) sample solution - blank. The specificity study was shown that there is no interference from the extracted blank and the extraction solvent at the retention time of analyte peak. The acetaminophen peak was pure. The Purity factor (999.998) was more than purity threshold (990.000).

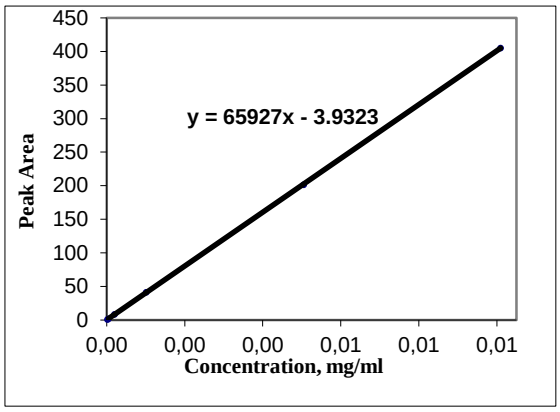

Fig. 1. Linearity curve (0.00002-0.01mg/ml).

Linearity and range. From standard solution of acetaminophen (0.01 mg/ml) the working solutions were prepared at six different concentration levels ranging from 0.00002 mg/ml to 0.01 mg/ml. Six replicate injections (n=6) were performed at each concentration. The linearity was checked by the correlation coefficient (acceptance criteria: >0.999), the square of correlation coefficient (acceptance criteria: >0.998), the RSD, % of retention times (acceptance criteria: <1.0 %). The calibration curve was constructed by plotting the response area against the corresponding concentration injected. The high value of the correlation coefficient indicates very good linearity. The linearity concentration and regression statistics are shown in Table 1. Fig. 1 shows the linearity curve.

Limit of quantitation (LOQ) and limit of detection (LOD). The limit of quantitation is estimated to be ten times the signal-to-noise ratio (s/N); the limit of detection is estimated to be three times of s/N ratio (acceptance criteria) according to USP. The quantitation limit was achieved by injecting a series of possible dilute solutions of the analyte and the precision was established at the quantitation level. The RSD, % of peak areas should not be more than 10 % (acceptance criteria). The LOQ of the method was estimated to be equal to 0.0002 mg/ml and 0.00002 mg/ml could be considered as the LOD according to the acceptance criteria.The s/N and RSD, % for LOQ - 27 and 4.9 % (n=6); for LOD — 3.5 and 9.6 % (n=6).

System suitability test. System suitability was checked by six replicate injections (n=6) of standard solution (0.01 mg/ml). The results are summarized in Table 2.

Standard solution stability. The stability was checked using two standard solutions (0.01 mg/ml) and by the calculated percentage difference between peak areas of standard solutions stored at room temperature and freshly prepared. Similarity factor between two standard solutions was within 0.98-1.02 (acceptance criteria) and the percentage difference between peak areas of standard solutions

Table 1.
The linear regression data

Level	Concentration, mg/ml	Average peak area	RSD of peak areas, % (n=6)
I	0.01	405.28	0.091
II	0.005	201.55	0.185
III	0.001	41.29	0.609
IV	0.0002	8.24	4.957
V	0.00004	1.90	6.804
VI	0.00002	0.90	9.626
Correlation coefficient (r)		0.99999	
Square of correlation coefficient (r^2)		0.99999	

stored at room temperature within 6, 24 and 48 hours and freshly prepared was less than 3.0 % (acceptance criteria). This gives the confidence that API residues are stable and the residues concentration do not change in sample solutions during the cleaning validation analysis.

Table 2.
The system suitability test results

Parameter	Acceptance criteria	Result
RSD of peak areas	<2.0 %	0.091 %
RSD of retention times	<1.0 %	0.024 %
Tailing factor	0.8-1.2	1.11
Theoretical plates	>2000	5878

Compatibility of the swab material. The compatibility of swab material (polyester) was checked using standard solution and extracted swab solution added standard at the same concentration. This parameter confirms that the swab material does not have the influence on determination of API residues and shows that swab material desorbs the analyte after sampling. The influence was evaluated quantitatively by the calculated percentage difference between peak areas obtained from the standard solution and the extracted swab solution added standard which should not be more than 3.0 % (acceptance criteria). The result is 0.02 %. Hence, the swab material does not effect on the determination and desorbs acetaminophen residues.

Estimation of acetaminophen residues in sample solutions. After manufacturing Ephact Flu capsules equipment cleaning samples were collected from different sampling points. The equipment surfaces were rinsed with deionized water for several times in order to remove extraction solution – diluent (ethanol). In laboratory the swab and rinse samples were tested immediately for estimation of acetaminophen residues using the developed and validated HPLC method. The analysis results are shown in Table 3. Fig. 2 shows the chromatogram obtained from the sample solution.

The determined concentration of residues of acetaminophen varies from 0.000024 mg/ml to 0.00022 mg/ml which is well below the calculated limits of cross-contamination. In spite of Ephact Flu capsules containing acetaminophen as the insoluble API in room temperature water from the point of view of cleaning validation cleaning standard operating procedure provides sufficient removal of the API residues from equipment surfaces and totally excludes the risk of cross-contamination of the next finished product.

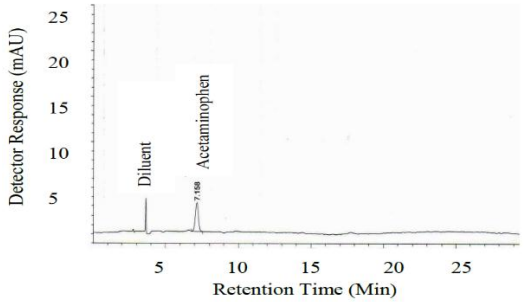

Fig.2. The chromatogram of sample solution

Table 3.
The results of analysis of acetaminophen residues in sample solutions

Sampling point #	Concentration, mg/ml
1	0.000222
2	0.000024
3	0.000115
4	<LOD
5	0.000099

Conclusion. Thus, the developed and validated selective and rapid HPLC method with appropriate swab and rinse sampling procedures and the methodology of establishing limits of cross-contamination can be used in pharmaceutical quality control laboratories to apply successfully in cleaning validation for quantitative estimation of acetaminophen residues and assay determination in pharmaceutical formulations.

ანალიზური ქიმია

ფარმაცევტული დანადგარის ზედაპირებზე აცეტამინოფენის ნარჩენების რაოდენობრივი განსაზღვრისა და სინჯის აღების მეთოდების შემუშავება და ვალიდაცია

ი. რუბაშვილი*, ნ. ქარუხნიშვილი**, ხ. მახარაძე**, ვ. ციციშვილი§

*ივანე ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის ფიზიკური და ორგანული ქიმიის ინსტიტუტი, თბილისი, საქართველო
**შპს ავერსი–რაციონალი, ხარისხის კონტროლის ლაბორატორია, თბილისი, საქართველო
§აკადემიის წევრი, ივანე ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის ფიზიკური და ორგანული ქიმიის ინსტიტუტი, თბილისი, საქართველო

წარმოდგენილ სტატიაში კვლევა ეხება ფარმაცევტული საწარმოო დანადგარების ზედაპირებიდან აღებულ ნიმუშებში აცეტამინოფენის ნარჩენების რაოდენობრივი განსაზღვრის სწრაფი და სელექციური მაღალეფექტური სითხური ქრომატოგრაფიული მეთოდის შემუშავებასა და ვალიდაციას, ასევე დასუფთავების ვალიდაციისთვის დასაშვები ზღვრების დადგენის მეთოდოლოგიის შემუშავებას. შემუშავებული ნიმუშის აღების პირდაპირი და არაპირდაპირი ჩამორეცხვის პროცედურები და მაღალეფექტური სითხური ქრომატოგრაფიული მეთოდი ვალიდირებულ იქნა სისწორის, სისტემის ვარგისობის ტესტის, სპეციფიკურობის, სწორხაზოვნობის, რაოდენობრივი განსაზღვრისა და აღმოჩენის ზღვრების პარამეტრების გამოყენებით. შემოწმებულ იქნა სტანდარტული ნიმუშის ხსნარების სტაბილურობაც. საკალიბრო მრუდი არის წრფივი r^2=99999 0,00002–0,01 მგ/მლ კონცენტრაციების დიაპაზონში; მეთოდის აღმოჩენის ზღვარია - 0,00002 მგ/მლ და რაოდენობრივი განსაზღვრის ზღვარი - 0,0002 მგ/მლ.

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