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Validation of an HPLC–UV method for the determination of digoxin residues on the surface of manufacturing equipment

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Abstract: In the pharmaceutical industry, an important step consists in the removal of possible drug residues from the involved equipments and areas. The cleaning procedures must be validated and methods to determine trace amounts of drugs have, therefore, to be considered with special attention. An HPLC–UV method for the determination of digoxin residues on stainless steel surfaces was developed and validated in order to control a cleaning procedure. Cotton swabs, moistened with methanol were used to remove any residues of drugs from stainless steel surfaces, and give recoveries of 85.9, 85.2 and 78.7 % for three concentration levels. The precision of the results, reported as the relative standard deviation (*RSD*), were below 6.3 %. The method was validated over a concentration range of 0.05–12.5 μg mL^{-1}. Low quantities of drug residues were determined by HPLC–UV using a Symmetry C18 column (150×4.6) mm, 5 μm) at 20 °C with an acetonitrile–water (28:72, v/v) mobile phase at a flow rate of 1.1 mL min^{-1}, an injection volume of 100 μL and were detected at 220 nm. A simple, selective and sensitive HPLC–UV assay for the determination of digoxin residues on stainless steel was developed, validated and applied.

Keywords: cleaning validation; digoxin; swab analysis; residues.

INTRODUCTION

An important step in the manufacture of pharmaceutical products is the cleaning of equipment and surfaces. The cleaning procedures for the equipment must be validated according to good manufacture practice (GMP) rules and guidelines. The main objective of cleaning validation is to avoid contamination between different productions or cross-contamination. This cleaning is verified by determining the amount of residues on surfaces involved in the manufacture process.

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Cleaning validation consists of two separate activities: the first is the development and validation of the cleaning procedure used to remove drug residues from manufacturing surfaces and the second involves the development and validation of methods for quantifying residuals from the surfaces of manufacturing equipment. Furthermore, many sampling points of the manufacturing facility and the manufacturing equipment have to be tested to verify the occurrence of contamination. For these reasons, an analytical method for residue monitoring should also be rapid and simple.[1]

The acceptable limit for residue in equipment is not established in the current regulations. According to the FDA, the limit should be based on logical criteria, involving the risks associated with residues of a determined product. The calculation of an acceptable residual limit, the maximum allowable carryover of active products in production equipment should be based on therapeutic doses, the toxicological index and a general limit (10 ppm). Several mathematical formulas were proposed to establish the acceptable residual limit.[1–7]

Digoxin (Fig. 1) is a cardioactive glycoside isolated from the leaves of *Digitalis lanata* and is the most frequently used drug in the treatment of congestive heart failure.[8] Numerous methods have been published for the quantitative determination of digoxin in raw materials,[9–13] tablets,[14–18] other solid dosage forms,[19] human plasma, urine, serum[20–23] and *Digitalis* leaves.[24,25]

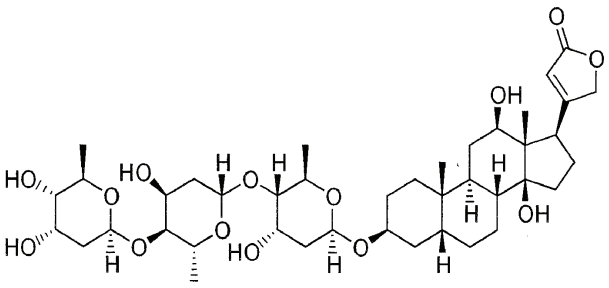

Fig. 1. Chemical structure of digoxin.

A literature survey revealed that no validation of cleaning methods for digoxin is to be found. Due to their high sensitivity and selectivity, analytical methods such as liquid chromatography were previously reported to be used for the determination of residues to control cleaning procedures.[5–7,26–32]

Taking the above-mentioned consideration into account, the aim of this study was to develop and validate a simple analytical method that allows the determination of trace levels of digoxin residues in production area equipment and to confirm the efficiency of the cleaning procedure. The analytical method reported was validated considering selectivity, linearity, accuracy, precision and limits of detection (*LOD*) and quantification (*LOQ*). The stability of digoxin samples was also studied.[33]

EXPERIMENTAL

Chemicals and reagents

The certified digoxin, working standard was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Acetonitrile and methanol (HPLC gradient grade) were purchased from J. T. Baker (Deventer, Holland). Purified water was obtained from Arium Laboratory Equipment (RO, UV) Sartorius AG (Gottingen, Germany). The extraction–recovery sampling was realized with Alpha® Swab polyester on a polypropylene handle – TX714A (ITW Texwipe®, Mahwah, NJ, USA). The mobile phase was filtered through a 0.45 µm Sartorius membrane filter (Gottingen, Germany).

Equipment

The HPLC system consisted of a degasser G1379B, a bin pump G1312A, an automatic injector G1329A, a thermostated column oven G1316A and a multiwavelength detector G1365B, all 1200 Series from Agilent Technologies, which were controlled by an HP Chemstation software (Waldbroon, Germany). The employed ultrasonic bath was from Elma, Transsonic 470/H (Singen, Germany) and the analytical balance was from Sartorius AG, CP224S – – OCE (Gottingen, Germany); accuracy of the balance: ± 0.0001 g.

Chromatographic conditions

All chromatographic experiments were performed in the isocratic mode. The mobile phase was acetonitrile-water (28:72, v/v) at a flow rate of 1.1 mL min^{-1}. The separation was performed at 20 °C on a Symmetry C18 column ((150×4.6) mm, 5 µm) from Waters (Milford, MA, USA). UV detection was realized at 220 nm. The injection volume was fixed at 100 µL.

Standard solutions preparation

The stock solution of standard was prepared by accurately weighing digoxin standard (approximately 12.5 mg) and transferring it into a 100 mL volumetric flask. Approximately, 20 mL of methanol was added and the content of the flask was sonified for 15 min. The solution in the flask was diluted to volume with 20 mL acetonitrile and water; the final concentration being 125 µg mL^{-1} digoxin. Dilutions were later prepared with water to obtain solutions for calibration (0.05–12.5 µg mL^{-1}) and standard solution for the positive swab control at three concentration levels (0.625, 3.125, and 15.625 µg per swab level). These solutions were filtered through a 0.45 µm regenerated cellulose filter before analysis and injected in triplicate.

Sample preparation

The selected surfaces (5 cm×5 cm) of stainless steel, previously cleaned and dried, were sprayed with 500 µL of a standard solution, for the positive swab control at all concentration levels, and the solvent was allowed to evaporate (approximate time was 2 h). The surfaces were wiped with the first cotton swab soaked with methanol, passing it in various directions, to remove the residues from the stainless steel. The other dry cotton swab was used to wipe the wet surfaces. The swabs were placed into a 25 mL screw cap test tube, and 5.0 mL of the purified water was pipetted into adequate sample tubes. The background control sample was prepared from the extraction media. The negative swab control was prepared in the same way as the samples, using swabs, which had not been in contact with the test surface. In addition, test and excipient solutions were prepared according to the content of tablets to assure that they did not interfere with digoxin. Subsequently, the tubes were placed in an ultrasonic bath for 15 min and the solutions were analyzed by HPLC–UV.

RESULTS AND DISCUSSION

Acceptance limit calculation

The maximum allowable carryover (MACO) is the acceptable transferred amount from the previous to the following product. The MACO is determined based on the therapeutic dose, toxicity and generally 10 ppm criterion. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the residue limit in terms of the contamination level of active ingredient *per* surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculations. The limit per surface area was calculated from the equipment surface area and the most stringent maximum allowable carryover (the most stringent criterion being based on the therapeutic dose in this case). The 0.1 % dose limit criterion is justified by the principle that an active pharmaceutical ingredient (API) at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects on human health. The calculated limit per surface area (LSA) in the case of digoxin was 1.20 μg swab^{-1} *pro* 25 cm^2. A stainless steel surface area of 5 cm×5 cm was chosen for practical reasons.

Optimization of the chromatographic conditions

To obtain the best chromatographic conditions, the wavelength for detection, the column and the mobile phase composition were adequately selected. The main objective was to develop an HPLC–UV method that, running in the isocratic mode, allowed the determination of digoxin residues collected by swabs, without interference of impurities originating from the swabs, plates and extraction media.

For the analysis, 220 nm was selected for detection because the drug has a sufficient absorption at this wavelength and low quantities of digoxin may be detected correctly. Furthermore, the calibration curve obtained at 220 nm showed good linearity.

Regarding the chromatographic procedure, different C18 columns were evaluated: LiChrosorb RP-18, Nucleosil C-18, μBondapak C-18, but Symmetry C-18 ((150×4.6) mm, 5 μm) was preferred to improve the peak symmetry and to obtain an appropriate retention time.

A mixture of acetonitrile–water in different proportions is frequently used as the mobile phase. In order to improve the selectivity and sensitivity, the amount of acetonitrile was varied between 20 and 40 vol. %. The best separation was achieved with the proposed mobile phase acetonitrile–water (28:72, v/v) at a flow rate of 1.1 mL min^{-1}. Furthermore, with decreasing flow rate, the column back-pressure is lower and hence the column life is prolonged.

The injection volume was varied between 10 and 100 μL and was finally set at 100 μL in order to increase the sensitivity of the method without sacrificing the chromatographic peak shape. Taking into account the results obtained with

different C18 columns and the tested mobile phases, the already defined chromatographic conditions were finally chosen. The sensitivity achieved by this method was much better than the initial method given by Song *et al.*,[9] which was modified in order to accommodate the requirements of trace analysis specific for a cleaning validation study.

Optimization of the sample treatment

Cotton swabs were spiked with different quantities of digoxin and placed into glass tubes. After the addition of different solvents (water, methanol and the mobile phase), the tubes were sonified for different times (5, 15 and 30 min) and the solutions analyzed by HPLC. The optimum conditions were achieved with water as the extracting solvent and a sonification time of 15 min. In all cases, the best results were obtained using two cotton swabs (the first wetted with methanol and the second dry); hence, this technique was applied in the subsequent work.

Validation of the method

Once the chromatographic conditions had been selected, the method was validated, whereby attention was paid to the selectivity, linearity, limit of detection, limit of quantification, precision, accuracy and sample stability.

Suitability test. System suitability testing is essential for the assurance of the quality performance of a chromatographic system. During performing the system suitability tests, in all cases, *RSD* of the peak areas was < 2 %, the average number of theoretical plates per column was > 5500 and the USP tailing factor was about 1.08.

Selectivity. The selectivity of the method was checked by injecting the digoxin standard, the background control sample, the negative swab control, the unspiked stainless steel 5 cm×5 cm plate swabbed as described and the excipient mixture (according to the content of the tablets). The results are shown in Fig. 2, from which it can be observed that there were no mutual interferences.

Linearity. Linearity data were obtained by plotting the area of the digoxin peak, expressed in area units, against the concentration of digoxin, expressed as μg mL^{-1}. A linear regression least square analysis was performed in order to determine the slope, intercept and coefficient of determination. The standard curve was linear from 0.05–12.5 μg mL^{-1}. The values of the slope, intercept and coefficient of determination of the calibration curve for digoxin are given in Table I. The high value of the coefficient of determination indicated good linearity.

Limit of detection and quantification. LOD and *LOQ* were determined based on the standard deviation of the response (*y*-intercept) and the slope of the calibration curve at low concentration levels according to ICH guidelines. The *LOD* and *LOQ* for digoxin were found to be 0.010 and 0.030 μg mL^{-1}, respectively.

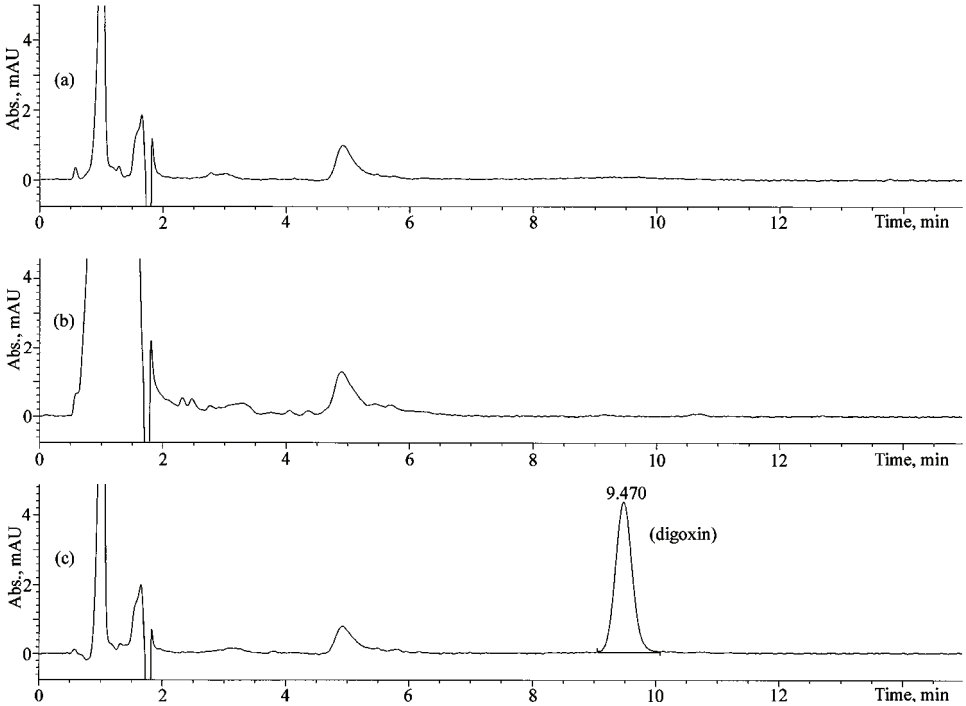

Fig. 2. Chromatograms obtained from: a) non-spiked stainless steel surface, b) excipient mixture and c) standard solution of digoxin (1.25 μg mL^{-1}).

TABLE I. Linear regression data in the analysis of digoxin

Statistical parameters	Values
Concentration range, μg mL^{-1}	0.050–12.50
Regression equation	$y = 64.342x + 4.7658$
Coefficient of determination	$r^2 = 0.9999$
$S(a)$-error in intercept	2.684
$S(b)$-error in slope	0.428
$S(y/x)$-error for y-est	0.195

Precision and accuracy. The precision and accuracy of the procedure, reported as relative standard deviation (*RSD*) and the recovery (%), respectively, were assessed by comparing the amount of analyte determined *versus* the known amount spiked at three different concentration levels with 6 replicates ($n = 6$) for each investigated concentration level. The method precision and accuracy were determined for spiked and dried swabs and plates. The recovery, 95 % confidence interval, and the *RSD* values obtained on spiked and dried swabs and plates (Table II) for each level illustrated the good precision and accuracy of the method. These precision and recovery results are acceptable for the purpose of residue monitoring.

TABLE II. Precision and accuracy of the results obtained from swabs and plates spiked with digoxin

Samples	Amount added μg mL^{-1}	Amount found μg mL^{-1}	95 % Confidence interval, %	Recovery %	*RSD* / % *n* = 6
Swabs	0.125	0.116	92.3–93.2	92.7	3.1
	0.625	0.580	89.8–95.2	92.5	3.6
	3.125	2.836	89.9–91.6	90.7	0.6
Plates	0.125	0.107	83.5–86.3	85.9	3.0
	0.625	0.530	80.9–89.5	85.2	6.3
	3.125	2.461	77.1–80.4	78.7	1.3

The intermediate precision of the method was investigated by performing five consecutive injections of standard solutions on two different days by different analysts. On both days, the *RSD* was calculated for peak area responses obtained for the digoxin peak. The data obtained suggested that the method exhibited an acceptable intermediate precision with less than 1.0 % *RSD* for the digoxin standard solution when analyzed on two different days by two different analysts.

Sample stability. The stability of the digoxin in the swab matrix was tested. The spiked samples at all concentration levels were stored after analyses in the injector vials in the auto-sampler tray at ambient temperature for 7 days. All the samples were injected into the appropriate HPLC system after 24 h, 48 h and 7 days against fresh standard solutions. The stability of the standard digoxin solution (1.25 μg mL^{-1}) was also inspected after storage for 7 days at ambient temperature and refrigerated. No changes in the chromatography of the stored samples were found and no additional peaks were registered when compared with the chromatograms of the freshly prepared samples (Table III).

TABLE III. Stability results obtained from digoxin swab extract samples

Sample (*n* = 3)	Mean recovery±*IC*[a], %			
μg per swab	0 h	24 h	48 h	7 days
0.625	92.2±1.7	93.5±1.8	92.6±1.0	93.2±0.6
3.125	90.2±1.3	90.6±1.5	90.7±0.8	90.1±0.8
15.62	88.9±1.2	89.3±0.8	89.3±1.1	88.9±1.3

[a]Interval of confidence

Assay of swab samples collected from different locations within the equipment train

Swab samples from different locations within the manufacturing equipment train were submitted to the laboratory for analysis of residual digoxin. These samples were prepared and analyzed by the proposed method. Some of the results obtained for these samples are presented in Table IV. An example of a chromatogram which originated from Glatt WSG (Bowl bottom mesh point), which is below the limit per surface area (LSA) and above the LOQ is shown in Fig. 3.

TABLE IV. Results obtained for the determination of digoxin in actual swab samples collected from 25 cm^2 swabbed area from different locations within the equipment train

Equipment swabbed	Location swabbed	Digoxin detected, µg per swab
Material dispensing scoops	Internal surface	<*LOQ*
	Back round plate	<*LOQ*
	External surface	<*LOQ*
High shear mixer – Diosna	Bottom of gran. bowl	<*LOQ*
	Chopper shaft	<*LOQ*
	Impeller blade	<*LOQ*
Turbo sieve – Bohle	Stainless steel inlet ring	0.24(<*LSA*[a])
	Product inlet, side wall	<*LOQ*
	Sieve unit	<*LOQ*
Fluid bed dryer – Glatt WSG	Inside wall	<*LOQ*
	Viewing window	<*LOQ*
	Bowl bottom mesh	0.87(<*LSA*)
Pillar hoist – FBD bowl inverter	Internal surface-top	<*LOQ*
	Internal surface-bottom	<*LOQ*
	Collar	<*LOQ*
Washer – extractor Miele	Drum back plate	<*LOQ*
	Drum perforated surface	<*LOQ*
	Door-middle	<*LOQ*
Metal detector – Lock Met 30+	In-feed chute	0.223(<*LSA*)
	Reject device, corner	<*LOQ*
	Reject flap	<LOQ
Deduster – Kramer	Inlet plate	0.277(<LSA)
	Dedusting helix	<*LOQ*
	Outlet	<LOQ
Tablet press – Kilian	The table	0.357(<*LSA*)
	Tablet chute cover	<*LOQ*
	Main gate	0.355(<*LSA*)

[a]Limit per surface area

CONCLUSIONS

In conclusion, a simple to use HPLC–UV method to quantify residues of the active pharmaceutical ingredient digoxin on swabs, in support of cleaning validation of pharmaceutical manufacturing equipment, was developed. Validation studies showed that the HPLC–UV method is selective, linear, precise and accurate. To extract the digoxin residues from the surfaces, a wipe test procedure using two cotton swabs is recommended. The recoveries obtained from the stainless steel surfaces were close to 80 % or higher and there was no interference from the cotton swab. Stability studies show that the digoxin swab samples are, at least, stable over the investigated 7 days. The overall procedure can be used as part of a cleaning validation program in the pharmaceutical manufacture of digoxin.

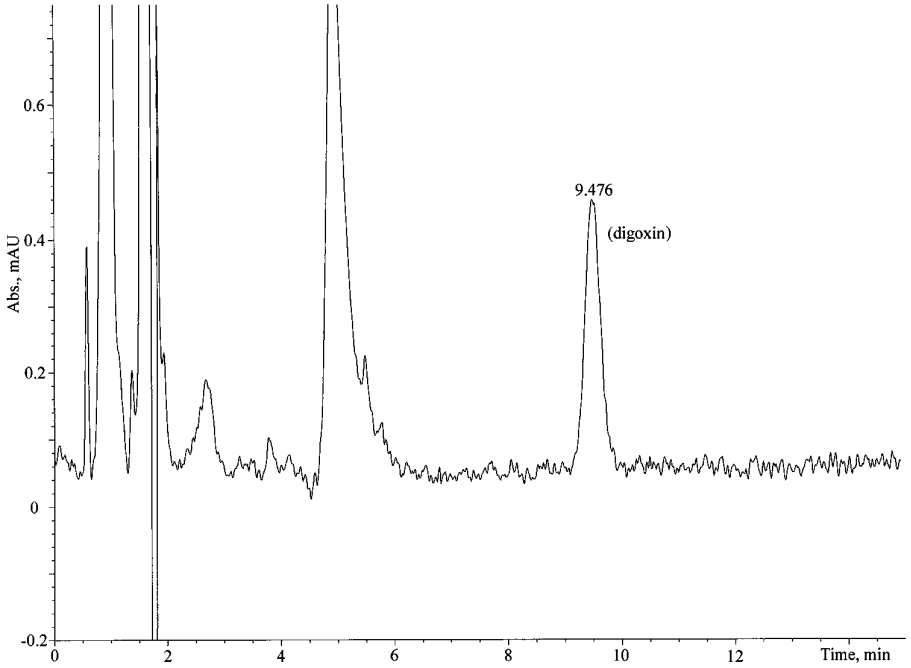

Fig. 3. Example of a chromatogram from a swab sample (Glatt WSG-Bowl bottom mesh point).

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ВАЛИДАЦИЈА HPLC–UV МЕТОДЕ ЗА ОДРЕЂИВАЊЕ ОСТАТАКА ДИГОКСИНА НА ПОВРШИНИ ПРОИЗВОДНЕ ОПРЕМЕ

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У фармацеутској индустрији, веома битан елемент представља уклањање могућих остатака активних супстанци са опреме и других површина укључених у производни процес. Процедура чишћења мора бити потврђена, те се стога мора посветити посебна пажња методи за одређивање остатака активних супстанци у траговима. Развијена је и потврђена HPLC–UV метода за одређивање остатака дигоксина на челичним површинама да би се контролисала процедура чишћења. Памучни брисеви, квашени метанолом, коришћени су за уклањање остатака дигоксина са челичне површине, а добијене вредности за препокривање износиле су 85,92, 85,22 и 78,74 %, на три концентрациона нивоа. Прецизност резултата, представљена као релативна стандардна девијација (*RSD*), била је испод 6,3 %. Метода је потврђена у опсегу концентрација 0,05–12,5 μg mL^{-1}. Мале количине остатака дигоксина одређене су помоћу HPLC коришћењем Symmetry C18 колоне ((150×4,6) mm, 5 μm), на 20 °C, са мобилном фазом ацетонитрил–вода (28:72, v/v) и протоком од 1,1 mL min^{-1}, инјекционом запремином од 100 μL и таласном дужином за детекцију од 220 nm. Коришћењем поменуте процедуре, развијена је, потврђена и примењена једноставна, селективна и осетљива HPLC–UV метода за одређивање остатака дигоксина на челичним површинама.

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