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A validated HPLC method for determining residues of a dual active ingredient anti-malarial drug on manufacturing equipment surfaces

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Abstract

Analytical method validation, determining the recovery rate from the equipment surface and the stability of a potential contaminant are important steps of a cleaning validation process. A rapid, sensitive and reproducible reversed-phase high-performance liquid chromatographic method was developed for the determination of pyrimethamine (PYR) and sulfadoxine (SUL) in cleaning validation swab samples. The active compounds can be selectively quantified in a sample matrix containing detergent and swab material as low as 0.12 μg/ml. The swabbing procedure used on stainless steel coupons was validated and the stability of PYR and SUL in the swab samples was assessed. The calculated limit of contamination values for PYR (4.99 μg/cm^2) and SUL (19.14 μg/cm^2) were not exceeded during four consecutive equipment cleaning trials. This confirms that the desired level of cleanliness is achieved with the current cleaning procedures, which are consequently validated.

Keywords: Cleaning validation; Cross-contamination; Anti-malarial drug; Pyrimethamine; Sulfadoxine; Reversed-phase liquid chromatography

1. Introduction

In the pharmaceutical industry it is of critical importance to establish documented evidence that cleaning procedures applied to the manufacturing equipment are able to remove residues to predetermined levels of safety. The validation of cleaning ensures that subsequent product batches are not contaminated by previously manufactured products or by the cleaning process itself. Much literature has been published on cleaning validation in the past 10 years [1–5] and official organizations (e.g., Pharmaceutical Inspection Convention and Food and Drug Administration) have regulated the subject through guidelines [6,7]. Still, there is limited practical guidance available. This article reports an approach for cleaning validation exemplified for a potent drug.

Challenges for cleaning validation are encountered especially when developing sensitive analytical methods capable of detecting traces of active pharmaceutical ingredients (APIs), which are likely to remain on the surface of the pharmaceutical equipment after cleaning [8,9]. The fact that some residuals are not detected by a method could mean that either the method is not sensitive enough for the residual in question or the sampling procedure is inadequate.

HPLC coupled with UV detection is widely used to monitor the efficiency of the cleaning methods due to its high sensitivity, selectivity and automation characteristics. All the compounds presenting chromophores (e.g., APIs, impurities and degradation products, placebo components, cleaning agents) whether they are hydrophilic or hydrophobic can be detected through HPLC-UV.

In non-dedicated manufacturing facilities, where there is a potential risk for cross-contamination, cleaning validation forms an integral part of the manufacturing process. One of the products that could cause the cross-contamination of

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other products manufactured with the same equipment is the anti-malarial drug Fansidar®. Fansidar tablets (Hoffman-La Roche, Basle, Switzerland) contain as active ingredients pyrimethamine (PYR) and sulfadoxine (SUL) in a weight ratio of 1:20. Both compounds present low water solubilities (PYR < 1 mg/ml and SUL < 0.1 mg/ml at 20 °C). PYR is more toxic than SUL (acute toxicity values: LD_{50} = 128 mg/kg oral, rat for PYR; LD_{50} = 5200 mg/kg oral, mouse for SUL). Due to its low water solubility and high toxicity, Fansidar® was chosen as the worst case among a range of products sharing several equipment pieces (a granulator, a wet mill, a fluid bed drier, a vacuum transfer system, a blender, a compression and a packaging machine).

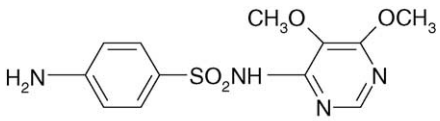
Pyrimethamine is a dihydrofolate reductase inhibitor, which is indicated in the treatment and prevention of parasitic diseases like malaria caused by *Plasmodium falciparium*, congenital toxoplasmosis caused by *Toxoplasma gondii*, meningeal leukaemia and against coccidiosis [10]. In malaria chemotherapy, pyrimethamine is combined in synergistic effect with sulfadoxine, a long-acting sulfonamide used for the treatment of bacterial infections. Fansidar acts by reciprocal potentiation of its two actives, producing a sequential blockade of two enzymes, namely dihydrofolate reductase and dihydropteroate synthetase, involved in the biosynthesis of folic acid within the parasites [11].

The independent determination of SUL through GC [12] and supercritical fluid chromatography [13] has been reported. Determination of PYR was achieved by fluorimetry [14], GC [15] and HPLC [16–18] in biological fluids, animal tissue and animal feeds. A variety of analytical methods for the simultaneous determination of both drugs in pharmaceutical preparations through spectrophotometry [19], in biological fluids through HPLC [10,20–25] and micellar electrokinetic chromatography in veterinary preparations [26] have been reported.

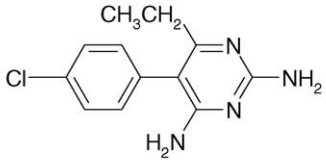
The simultaneous determination of PYR and SUL is impeded by the differences in their chemical properties. PYR is a weak base (pK_a = 7.3) and SUL is an ampholyte (pK_1 = 1.8; pK_2 = 6.1) [20]. The analysis is even more complicated by the presence in the sample of the detergent components. The detergent contains sodium xylene sulfonate (SXS) with acidic properties (acute toxicity value: LD_{50} = 1000 mg/kg oral, rat). A method for analyzing all three components has not yet been reported. The chemical structures of these compounds are shown in Fig. 1.

The aim of this study was to validate the cleaning procedures used for the manufacturing equipment involved in Fansidar manufacture. Three tasks were identified:

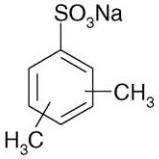
- development and validation of a sensitive chromatographic method capable of detecting traces of PYR, SUL and SXS likely to remain on pharmaceutical manufacturing equipment surfaces after cleaning;
- development and validation of a suitable sampling method;
- testing the efficiency of the current cleaning procedures.



Sulfadoxine



Pyrimethamine



Sodium Xylene Sulfonate

Fig. 1. Chemical structures of sulfadoxine, pyrimethamine and sodium xylene sulfonate.

2. Materials and methods

2.1. Equipment

The HPLC system was an Alliance Waters 2690 (Mildford, MA, US) equipped with Waters 996 photodiode array detector and a detection cell of 8 μl. Waters Millenium software (Version 3.20) was used for data acquisition and processing.

2.2. Chemicals and reagents

All chemicals were of analytical grade. HPLC grade methanol was purchased from Lab-Scan (Dublin, Ireland). *ortho*-Phosphoric acid 85% (v/v) was purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1 M) Fixanal® (Riedel-de-Häen), and SXS (Aldrich) were purchased from Sigma–Aldrich (Johannesburg, South Africa). Sulfadoxine (*N′*-(5,6-dimethoxy-4-pyrimidinyl)sulfanilamide) and pyrimethamine (5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine) certified standards were supplied by Roche Diagnostics (Mannheim, Germany). Detergent was purchased from Health & Hygiene (Johannesburg, South Africa). Solutions were prepared with deionized water, produced by a MilliQ water purification system from Milipore (Bedford, MA, US). The sampling was performed with Bemcott M_3 wipers purchased from Asahi Chemical Industry (Japan). Samples were filtered through PVDF Millex HV-25 syringe filters purchased from Microsep (Johannesburg, South Africa).

2.3. Chromatographic conditions

Chromatographic separations were performed on a Waters 150 mm × 4.6 mm Symmetry 5 μm, C18 analytical column, 100 Å pore diameter.

The chromatographic experiments were performed under isocratic elution. The mobile phase consisted of methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v). The mixture was allowed to cool down to 20 °C in a water bath and it was adjusted to pH* 3.0 with 1 M sodium hydroxide. The mobile phase was degassed by sonication under low vacuum prior to use. The flow-rate was set to 1.0 ml/min. The autosampler temperature was 15 °C and the column temperature was set at 26 °C. The injection volume was 50 μl. The detector was programmed to record the absorbance of the samples between 190 and 300 nm with a sampling frequency of 1 spectrum/s. The raw data acquired with the photodiode array detector were processed at 270 nm.

2.4. Preparation of calibration solutions

A stock solution containing approximately 250 μg/ml PYR and 250 μg/ml SUL was prepared in methanol. Intermediate stock solutions with concentrations ranging between 5 and 200 μg/ml were prepared in methanol at seven concentration levels. The final dilutions were prepared as follows: 1 ml of 0.1 M urea solution was spiked with 0.2 ml methanolic intermediate stock solution and the solution was made up to 10 ml with mobile phase. The concentrations of the calibration solutions are: 0.10, 0.20, 0.50, 1.01, 2.02, 3.03, 4.03 μg/ml for PYR and 0.10, 0.20, 0.51, 1.02, 2.03, 3.05 and 4.07 μg/ml for SUL.

2.5. Sample preparation

Bemcott M_3, a non-woven, entangled fabric made of continuous filaments of 100% cellulose, fabricated without any adhesive binders was selected for its highly absorbent properties. Three swabs cut in pieces of 2 cm × 2 cm were placed into 15 ml test tubes. The swabs were spiked with various quantities of active compounds resulting in final solutions with the same theoretical concentrations as for the standard calibration solutions when diluted with 10 ml of solvent. The swabs were left to dry. Then, 1 ml of 0.1 M urea solution and 9 ml of mobile phase was added. The test tubes were vortexed for ca. 30 s and then the solution was filtered through PVDF (0.45 μm) syringe filters, discarding the first 2 ml of the solution. Four replicates per level were prepared as described above.

Three replicate blank samples for the swab material were prepared as above.

A detergent sample was prepared by diluting 200 mg of detergent concentrate into 100 ml MilliQ water and further diluting 5 ml of this solution with mobile phase in a 20 ml volumetric flask. The final detergent solution has a concentration of 12.5 μg/ml SXS. An independent standard solution for SXS was prepared in order to identify the SXS peaks obtained for the detergent solution.

3. Results and discussion

3.1. Method optimization

A proprietary method, which was developed, validated and approved for Roche's affiliates, was re-evaluated. This initial method, which is used to assay PYR and SUL in pharmaceutical formulations at relative high concentrations, was modified in order to accommodate the requirements of trace analysis specific for a cleaning validation study.

A Symmetry® column was used to improve the peak symmetry and obtain the best overall chromatographic separation of the cleaning validation samples.

The initial method detects PYR and SUL at 227 nm. For tablet content assay, an optimum simultaneous detection of both components is ensured at this wavelength where the much lower absorption of SUL is compensated by the high content of SUL per tablet. The UV spectra of PYR and SUL in the mobile phase show stronger absorption maxima for both compounds around 270–273 nm.

For the cleaning validation assay, the detection was performed at 270 nm in order to improve the method sensitivity towards SUL which is less soluble than PYR and therefore likely to remain in higher quantities on the pharmaceutical equipment after cleaning. At this wavelength possible interferences from the detergent and other sample compounds are minimized.

The injection volume was increased from 20 μl, in the initial method, to 50 μl in order to enhance the method sensitivity.

3.2. Selectivity

The solutions for detergent and for the active components were chromatographed on the Symmetry column but maintaining the mobile phase from the initial method. It was observed that the detergent components are interfering with the active components of the drug. A peak was also observed when solutions prepared for the swab blank were injected.

Several parameters of the mobile phase were simultaneously optimized in such a way until baseline resolution was obtained for all the peaks. The final optimum separation conditions were achieved when the mobile phase composition was methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v) adjusted to pH* 3.0 with 1 M sodium hydroxide.

With the optimized separation conditions, HPLC runs with detergent solution and swab blanks indicated that no swab or detergent component is detected at the retention time of SUL and PYR. Fig. 2 shows separation profiles of the samples injected to assess the selectivity of the method.

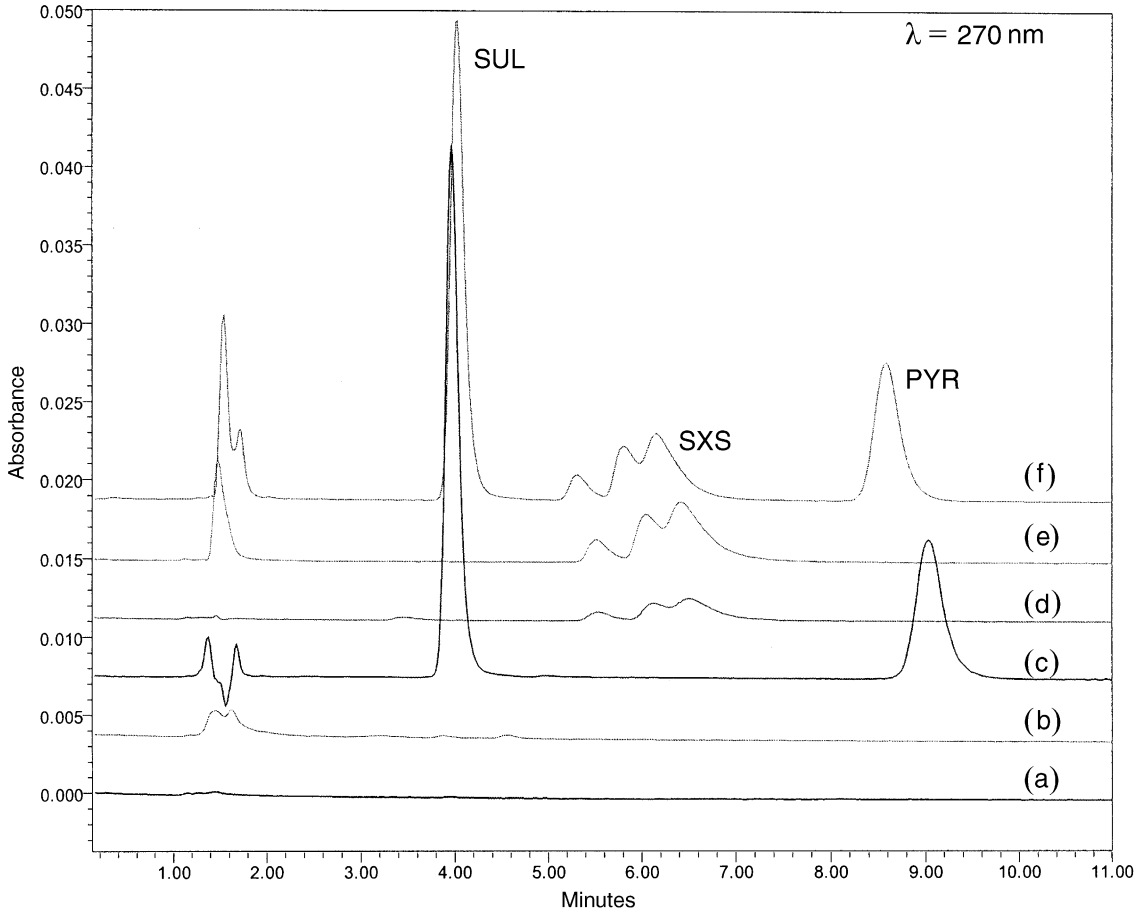


Fig. 2. Typical chromatograms for: mobile phase (a), an extracted swab blank (b), the chromatogram obtained for the 100% level (c), an SXS solution (d), a detergent solution (e) and a combined sample containing detergent, swab and the drug actives (f).

SXS is a mixture of five constitutional isomers. With the current separation conditions it elutes as a group of three peaks in the time interval from 5 to 7 min. SUL and PYR are eluting at 4.2 and 8.6 min, respectively. The retention time of PYR in Fig. 2f was slightly earlier than in Fig. 2c probably due to matrix differences. The identity of PYR in Fig. 2c was confirmed by the UV spectrum. The complete sample separation was achieved within 10 min.

3.3. Method range

The new method was developed for the concentration range of interest for cleaning validation, unlike the case of the methods, which are validated for APIs assay on a relatively small concentration range from 80 to 120% of the expected concentration. For cleaning validation, a wider concentration range with at least five concentration levels should be validated. This is due to the fact that it is difficult to predict the actual level of contaminants found on the production equipment and to avoid re-diluting and re-injecting too concentrated samples.

3.4. Linearity

Linearity was studied for swab samples (seven concentration levels; four replicates per level). Linearity data were obtained by plotting the peak area of PYR and SUL expressed in area units against the concentration of PYR and SUL expressed as μg/ml. A linear regression least square analysis was performed in order to determine the slope, intercept and correlation coefficient of the standard curve.

Standard curves were linear from 0.129 to 4.02 μg/ml PYR and from 0.120 to 4.06 μg/ml SUL. The coefficients of correlation (r) are higher than 0.999 for both PYR and SUL indicating a good relationship between the peak area and the concentration of PYR and SUL, respectively in these concentration ranges.

The intercepts of the regression lines are situated for both PYR and SUL within the 95% confidence band of ±10.0% of $\langle Y \rangle_{\mathrm{Ref}}$ indicating that there are no constant systematic errors.

The limits of detection and quantification were calculated with the following formulas:

$$\mathrm{LOD} = \frac{3.3s}{S}, \qquad \mathrm{LOQ} = \frac{10s}{S} \tag{1}$$

Table 1
Parameters of linearity for pyrimethamine and sulfadoxine

Analyte	Calibration range (μg/ml)	*y*-intercept	Slope	r^2	LOD (μg/ml)	LOQ	
						μg/ml	μg/swab
PYR	0.042–4.020	−2.78E+03	1.030E+05	0.9987	0.042	0.129	1.29
SUL	0.040–4.060	−3.391E+03	2.198E+05	0.9989	0.040	0.120	1.20

Table 2
Intra-assay precision (1 day)

Concentration level (%)	%RSD ($n = 4$)	
	PYR	SUL
5	2.1	1.5
10	1.3	1.9
25	2.4	2.2
50	1.6	1.4
100	2.4	2.7
150	1.8	1.0
200	3.3	3.2
Average	2.1	2.0

where *S* is the slope of the calibration curve and *s* the standard deviation. For the estimation of the standard deviation *s*, the standard deviation of the *y*-intercept was used. The linearity data are presented in Table 1.

3.5. Precision (by repeatability)

The intra-assay precision of the chromatographic method, reported as relative standard deviation, was assessed by measuring the repeatability of the results obtained for four replicate swab samples at seven concentration levels. Swabs were spiked with various quantities of analyte, allowed to dry and then extracted in mobile phase. The %RSD values obtained per level are presented in Table 2.

The overall precision expressed as %RSD is 2.1 for PYR and 2.0 for SUL.

3.6. Precision (by reproducibility)

The inter-assay precision was assessed for three concentration levels (50%, 100%, 150%) with six replicate swab samples per level (see Table 3). The assays were carried out over 3 days on the same instrument by one operator ($n = 18$).

These values demonstrate that the precision of the method is adequate over the range of concentrations expected in cleaning validation samples.

Table 3
Inter-assay precision (3 days)

Concentration level (%)	%RSD ($n = 18$); 3 days	
	PYR	SUL
50	3.2	4.8
100	4.3	1.7
150	2.9	2.3
Average	3.5	2.9

3.7. Accuracy (by recovery)

The method accuracy was determined on spiked and dried swabs that where then extracted in mobile phase. The accuracy of the procedure was assessed by comparing the analyte amount recovered from swabs versus the known amount in the standard injection solutions at seven concentration levels with four replicates ($n = 4$) for each concentration level investigated.

Recoveries from swabs higher than 100% were obtained especially for SUL (Table 4). This was seen as a positive systematic error caused by the low SUL solubility. Various methods were used in order to increase the solubility of SUL (data not shown). It was observed that a solution of 0.1 M urea, when added to the samples, increases the SUL solubility. It is assumed that urea neutralizes the hydrogen bonds between the SUL molecules, therefore preventing their association and precipitation phenomena.

In the case of spiking swabs with SUL it is believed that the cotton acts as a "buffer" medium between the methanolic standard solution containing SUL and the aqueous mobile phase, therefore preventing SUL precipitation. However, precipitation is more readily to occur for the samples prepared without cotton swabs.

3.8. Establishing limits for the cross-contamination level permitted on clean equipment

It is to be remarked that contamination refers to any chemical, microbiological or particulate contaminant likely to remain on the equipment surface after its cleaning, whereas cross-contamination refers to the contamination of a product by a previously manufactured product.

The maximum permitted quantities (*R*) of PYR and SUL as potential cross-contaminants were calculated through several methods [27] (see Table 5). The total surface area of the equipment chain in direct contact with the product was ac-

Table 4
Accuracy by recovery

Concentration level (%)	Recovery (%) ($n = 4$)	
	PYR	SUL
5	96.6	127.6
10	103.2	136.9
25	108.2	124.3
50	115.1	126.4
100	102.7	106.9
150	99.9	103.6
200	100.2	102.7

Table 5
Maximum permitted levels of cross-contamination obtained through two different calculation methods

Criterion	Permitted cross-contamination (μg/cm^2)	
	PYR	SUL
0.1% dose limit	4.99 (LOC)	39.88
10 ppm	19.14	19.14 (LOC)

counted for in the calculations. The lowest obtained values were selected as limit of contamination (LOC) for this study.

A lowest calculated value of 4.99 μg PYR/cm^2 was obtained when the 0.1% dose limit criterion was used for the total equipment chain. The 0.1% dose limit criterion is justified by the principle that an API at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects to one's health. This criterion as explained by [27] accounts also for the maximum daily intake of a following product and for the batch size of the product that will be manufactured next with the same equipment (see Eq. (2))

$$R = \frac{DS}{IF}\frac{1}{A}\ (\mu g/cm^2) \quad (2)$$

For SUL, the lowest calculated value was obtained when the 10 ppm acceptance criterion was applied. When not more than 10 ppm of SUL were allowed into the next manufactured product, a limit of 19.14 μg SUL/cm^2 was determined as LOC (see Eq. (3))

$$R = 10 \times S\frac{1}{A}\ (\mu g/cm^2) \quad (3)$$

where R is the maximum residue of API permitted after cleaning, allowed into the next product; it is assumed that the total amount of residue is distributed homogenously into the following product; D the lowest daily therapeutic dose of the contaminant; S the lowest batch size of the product to follow; I the maximum daily intake of the product to follow; F the safety factor (can vary from 10 to 100 000 depending on the product nature, e.g., topical, oral or injectable preparations); A the total surface area of equipment in direct contact with the products, calculated based on the assumption that all the products come into contact with all the equipment pieces of the chain.

3.9. Determination of recovery rate of contaminants from stainless steel

Recovery studies were performed in order to determine to what extent the residue could be retrieved from the production equipment with the sampling procedure chosen. The recovery experiment was performed at the LOC level of PYR and SUL on the equipment.

PYR and SUL were independently spiked on 316 stainless steel coupons (10 cm × 10 cm). The spiked coupons were allowed to dry (ca. 2 h) at room temperature. Since the swabbing is an operator dependant technique, each analyte was recovered six times from coupons in order to obtain a representative mean of the recovery rate. The swab sampling simulation was performed by one operator.

A pair of tweezers was used to perform the swabbing. Various proportions of water–methanol were considered as swabbing solvents. A mixture of methanol–MilliQ water 70:30 (v/v) swabbing solvent provided the highest recovery rate. Better recovery results were obtained when the spiked analytes were recovered by using three swabs per sample rather than two. The first swab was wetted with 0.2 ml of swabbing solvent. The coupon was swabbed horizontally with one side of the swab and vertically with the other side. The procedure was repeated two more times with two more wetted swabs. All three swabs were collected into the same test tube. The swab samples were prepared as described above. Appropriate dilutions were performed in order to bring the concentration of the samples within the validated range of the analytical method. Table 6 presents the recovery results.

The recovery experiment provided a good indication regarding the reproducibility of the swabbing procedure. RSD values of 7.4% for PYR and 5.1% for SUL were obtained ($n = 6$).

3.10. Sample stability

The stability of the APIs in the swab matrix was tested. Several series of samples were prepared by spiking swabs with a quantity of analyte that would result in the nominal concentration level when diluted with 10 ml solvent. The first lot ($n = 4$) of samples were stored in a moistened state. The second lot ($n = 4$) was kept in a dry state. All samples were stored in amber test tubes, in the refrigerator (4 °C) for 7 days. It is known that a methanolic stock solution containing 5.0 mg SUL/ml and 0.5 mg PYR/ml which is stored in amber glassware is stable at 4 °C for over 6 months [28]. Before analysis the samples were allowed to reach room temperature. Urea solution 0.1 M (1 ml) and 9 ml of mobile phase was added to the dry stored samples. A fresh sample was prepared on the day of the analysis. All the samples were then vortexed, filtered through PVDF (0.45 μm) syringe filters and analyzed.

Table 6
Recovery of PYR and SUL from stainless steel coupons

Quantity of contaminant spiked	Recovery rate (%)	Mean recovery (%) ± IC (%)	%RSD ($n = 6$)
Pyrimethamine, 498.5 μg	96.4 81.2 84.8 93.9 97.9 88.8	90.5 ± 6.5	7.4
Sulfadoxine, 1880.7 μg	84.8 84.4 83.2 80.7 91.3 79.0	83.9 ± 4.2	5.1

Table 7
Sample stability (samples kept at 4 °C in the dark for 7 days)

Contaminant	Concentration (μg/ml)	Sample storage conditions	Recovery (%)	%RSD ($n = 4$)
PYR	2.01	Dry	95.8	1.3
		Soaked	96.7	1.7
SUL	2.02	Dry	97.9	1.4
		Soaked	98.0	1.8

Table 8
Results obtained for the determination of PYR and SUL in actual swab samples collected from the manufacturing equipment chain

Processing step	Contaminant (μg/cm^2)									
	Pyrimethamine (LOC = 4.99 μg/cm^2)					Sulfadoxine (LOC = 19.14 μg/cm^2)				
	Run 1	Run 2	Run 3	Run 4	Mean + 2s	Run 1	Run 2	Run 3	Run 4	Mean + 2s
Granulation	0.197	2.038	0.302	0.164	2.496	0.583	4.724	0.286	0.179	5.831
Milling	0.020	0.057	0.063	0.059	0.090	0.355	0.173	0.634	0.136	0.779
Drying	0.476	0.250	0.218	0.162	0.552	11.164	1.264	4.543	0.882	13.983
Blending	0.004	0.055	0.056	0.055	0.094	0.131	0.085	0.166	0.084	0.196
Vacuum transfer	0.007	0.041	0.059	0.040	0.080	0.098	0.252	0.166	0.157	0.295
Compression	0.010	0.050	0.081	0.050	0.106	0.167	0.174	1.287	0.128	1.570
Packing	0.077	0.327	0.087	0.072	0.389	3.275	0.837	0.481	0.113	4.036

No changes in the chromatography of the stored samples were found and no additional peaks appeared when compared with chromatograms of freshly prepared samples. It was observed that SUL is slightly more stable than PYR in swab samples. With each compound, similar recoveries were obtained for both dry and moistened stored samples and it was decided that samples should be stored in a dry state in order to avoid the double preparation of the mobile phase (see Table 7).

3.11. Assay of swab samples collected from the equipment train

Swab samples collected from different locations of the manufacturing equipment train were analyzed with the new method. The results found are presented in Table 8.

Since the hand cleaning is generally less reproducible than the automated cleaning, the validation results obtained for manual cleaning procedures must be consistent and well below the LOC. This is to ensure that residues will be removed efficiently and below the acceptance levels in the conditions of routine cleaning of the manufacturing equipment.

For the current study it was observed that all data obtained lie within 2*s* of the sample mean and well below the LOC. This gives the confidence that the manual cleaning procedures tested do provide sufficient removal of the residues from the equipment chain.

3.12. The place of the analytical method within a cleaning validation strategy

A successfully validated analytical method, sensitive enough for the level of contamination determined by calculating acceptance criteria, and supported by an appropriate sampling method for which validated recovery studies are established constitute the answers to the question often encountered in the cleaning validation literature of "How clean is clean" [29,30]. The analytical method measures the level of cleanliness of the equipment at the time of validation and monitors any trends and deviations from the validated status of a cleaning process susceptibly to develop in time.

4. Conclusion

An accurate, reproducible, sensitive and selective HPLC method for the simultaneous determination of PYR and SUL residuals on pharmaceutical manufacturing production surfaces has been validated to control the efficiency of the equipment cleaning. The chromatographic separation is achieved within 10 min facilitating a high sample throughput. The level of contamination found after equipment cleaning was monitored during several consecutive runs. The results obtained confirm that the cleaning procedures used are able to remove residues from equipment surfaces well below the calculated limit of contamination.

Acknowledgments

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References

[1] PDA Technical Report No. 29: Points to Consider for Cleaning Validation, PDA J. Pharm. Sci. Technol. 52 (1998) 1–23.

[2] R.Ch. Hwang, Pharm. Technol. 24 (2000) 62–67.
[3] D.A. LeBlanc, Pharm. Technol. 23 (1998) 136–148.
[4] T.M. Rossi, R.R. Ryall, in: C.M. Riley, T.W. Rosanske (Eds.), Development and Validation of Analytical Methods, Pergamon Press/Elsevier, New York, 1996, pp. 293–302.
[5] F. Laban, et al., S.T.P. Pharma Pract. 7 (1997) 87–127.
[6] Guide to Inspections Validation of Cleaning Processes, Food and Drug Administration, Office of Regulatory Affairs, Washington, DC, 1993, pp. 1–6.
[7] PIC/S, Recommendations on validation master plan, installation and operational qualification, non-sterile process validation, cleaning validation, PI 006-2, July 2004.
[8] M.J. Nozal, J.L. Bernal, L. Toribio, M.T. Martin, F.J. Diez, J. Pharm. Biomed. Anal. 30 (2002) 285–291.
[9] J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, J. Pharm. Biomed. Anal. 23 (2000) 421–428.
[10] C. Midskov, J. Chromatogr. 308 (1984) 217–227.
[11] A.H. Fairlamb, Parasitology 99 (1989) 93–112.
[12] C. Midskov, J. Chromatogr. 306 (1984) 388–393.
[13] S.I. Bhoir, I.C. Bhoir, A.M. Bhagwat, M. Sundaresan, J. Chromatogr. B 757 (2001) 39–47.
[14] P. Parimo, Indian J. Pharm. Sci. 50 (1998) 114–117.
[15] J.R. Harris, G. Baker, J.W. Munday, Analyst 102 (1977) 873–876.
[16] K. Bangchang, P. Tan-ariya, R. Ubalee, B. Kamanikom, J. Karbwang, J. Chromatogr. B 689 (1997) 433–437.
[17] M.D. Coleman, G. Edwards, G.W. Mihaly, J. Chromatogr. 308 (1984) 363–369.
[18] S. Horii, M. Nishi, N. Oku, H. Miyakawa, M. Tezuka, J. AOAC Int. 84 (2001) 1031–1034.
[19] J.O. Onah, J.E. Odeiani, J. Pharm. Biomed. Anal. 30 (2002) 851–857.
[20] H. Astier, C. Renard, V. Cheminel, O. Soares, C. Mounier, F. Peyron, J.F. Chaulet, J. Chromatogr. B 698 (1997) 217–223.
[21] M.D. Green, D.L. Mount, H. Nettey, J. Chromatogr. B 767 (2002) 159–162.
[22] Y. Bergqvist, S. Eckerbom, H. Larsson, M. Malekzadeh, J. Chromatogr. 571 (1991) 169–177.
[23] M.D. Edstein, I.D. Lika, T. Chongsuphajaisiddhi, A. Sabchareon, H.K. Webster, Ther. Drug Monit. 13 (1991) 146–151.
[24] V.K. Dua, R. Sarin, V.P. Sharma, J. Pharm. Biomed. Anal. 12 (1994) 1317–1323.
[25] J. Eljaschewitsch, J. Padberg, D. Schuermann, B. Ruf, Ther. Drug Monit. 18 (1996) 592–597.
[26] J.J. Berzas, G.C. Penalvo, F.J. Guzman, J. Liq. Chrom. Rel. Technol. 22 (1999) 1975–1986.
[27] G.L. Fourman, M.V. Müllen, Pharm. Technol. 17 (1993) 54–60.
[28] M. Edstein, J. Chromatogr. 305 (1984) 502–507.
[29] R.Ch. Hwang, D.L. Kowalski, J.E. Truelove, Pharm. Technol. 23 (1997) 62–68.
[30] I. Krull, M. Swartz, LC-GC 17 (1999) 1016–1019.