Validation of cleaning of pharmaceutical manufacturing equipment, illustrated by determination of cephradine residues

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The systematic approach developed to assess the amount of residues left on manufacturing equipment surfaces from product carryover is known as cleaning validation. Current trends have seen increasing demand for rapid sample analysis time along with low detection limits for verification of cleaning validation samples. A total organic carbon (TOC) method is sensitive to the ppb range and is less time consuming than high performance liquid chromatography (HPLC). The purpose of this study is to demonstrate how to develop and validate a TOC method for cleaning applications. Validation of the cleaning procedures for manufacturing or processing equipment has been presented in this paper. A sensitive and reproducible method was developed and validated for the determination of cephradine in swab samples. The method for determining residues of cephradine on manufacturing equipment surfaces was validated for precision, linearity, accuracy, limit of quantification and % recovery of a potential contaminant. The sampling procedure using cotton swabs was also validated. A mean recovery from stainless steel plate close to 78% was obtained. The assay was linear over the concentration range of 30 to 600 ng ml^{-1} concentration ($R \approx 0.9987$). The calculated limit of contamination value was less than 315 μg cm^{-2}, during three consecutive cleaning trials.

1. Introduction

Pharmaceutical products are very much susceptible to contamination from shared manufacturing equipment. In many cases, the same equipment may be used for processing different products.[1] Cleaning validation is the process of assuring that cleaning procedures effectively remove residue from manufacturing equipment below a predetermined level. This is necessary to assure the quality of upcoming product using the same equipment, to prevent cross-contamination. Good manufacturing practice in pharmaceutical manufacturing plants states that the equipment must be maintained in a clean and orderly manner.[2–8] Mostly, cleaning validation samples have been measured using high performance liquid chromatography (HPLC) methods, which are often time consuming and subject to a number of interferences. Total organic carbon (TOC) analysis is a new method which has previously been applied to only measurement of carbon residues on production surfaces for pharmaceutical equipment and for water quality checking. We have applied the TOC analysis method to examine the cephradine residue. This developed and validated method offers extremely low detection capability in parts per billion (ppb), rapid sample analysis time and therefore quick turn-around of production, equipment and facilities. The method allows the measurement of extraneous materials such as process intermediates and cleaning agents, which are not possibly detected by other non specific or specific methods. TOC for cleaning validation has several advantages over specific methods. Only one method is needed for all cleaning validation analysis, the method is simpler to implement and easier to validate than chromatographic techniques and less time consuming. The method always produces a "worst-case" result, assuming that all residues are the active substance. TOC analysis demonstrated the better correlation to cleaning validation compounds in comparison to traditional analytical methods. Some qualities that make TOC a viable part of a cleaning validation include: high sensitivity, high recovery of samples, non-specific measurement and ease of use, minimal interferences and cost effectiveness. Cost savings could be attained by using cleaning validation studies. For example, by reducing a 12 h cleaning turnaround time to 6 h, per day savings on all batches could be achieved. Cephradine was chosen for cleaning, it is a cephalosporin product manufactured by most of the pharmaceutical industries. This product can cross-contaminate the other running products, which are being manufactured using the same equipment pieces like cone blender, grall mixer, encapsulation machine, blistering machine and packaging machine *etc*. As far as the cleaning process is concerned, cephradine has been selected due to its low water solubility and high toxicity value. This method depends on various parameters like surface type (stainless steel, glass, vinyl),[9–13] and it was necessary to establish the way of addition of the drug on different surfaces and procedures to collect the sample.[14,15] TOC analysis involves the oxidation of carbon and the detection of the resulting carbon dioxide. A number of different oxidation techniques exist, including photocatalytic oxidation, chemical oxidation, and high-temperature combustion.

2. Experimental

2.1 Chemical and reagents

Cephradine reference standard was provided by Bristol Myers Squibb Pharmaceuticals, TOC grade water was prepared by

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Multi column distillation plant (Spirax ultra Pure System, USA). Phosphoric acid and sodium persulafte were purchased from Scharlau (Barcelona, Spain). Absorband TX762 absorbent cotton swabs were from Texwipe (Upper Saddle River, NJ).

2.2 Instrumentation and methodology

The development of this method and validation were performed on a Anatel A-2000 wide range TOC Analyzer. It measures TOC directly by adding phosphoric acid to the sample to reduce pH to approximately 2 to 3. At this low pH, any inorganic carbon that is present is liberated as CO_2 into a nitrogen carrier gas and is directly measured by a non-dispersive infrared (NDIR) detector. Any remaining carbon in the sample is assumed to be TOC. A sodium persulfate oxidant is then added to the sample, and in the presence of UV radiation, the remaining carbon is oxidized to CO_2. The amount of CO_2 generated is then measured by NDIR to determine the amount of TOC originally present in the water.

2.3 Sample preparation

The TOC swabbing performs the swabbing procedure as follows. An aliquot of 20 ml TOC grade water into 20 ml TOC vial. Desorb a polyster tipped swab in TOC vial containing 20 ml TOC grade water. Using one side of the moistened swab, swab 100 cm^2, moving from left to right and pour into TOC vial containing 20 ml TOC grade water. Using dry swab and perform additional swabbing on the same sampling area without desorbing into the water. The sealed TOC vial is vortexed for 10 s and analyzed for TOC.

3. Method validation

Linearity was tested using standard calibration curve at a concentration range of 30 to 600 ng ml^{-1}. These standards were tested six times in agreement to ICH guidelines.[16] A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding statistical study (ANOVA) ($p < 0.05$).[17] The accuracy was evaluated by the recovery of cephradine (300 ng ml^{-1}) at three different levels (150 ng ml^{-1}, 300 ng ml^{-1}, and 450 ng ml^{-1}), each level tested three times. The swabbing recovery study, which involved spiking cephradine on 100 cm^2 316 L stainless steel coupons, allowing the coupons to dry, recovering the cephradine with swabs, and desorbing the swabs into TOC grade water. These swabbing samples were then analyzed for TOC. Swabbing recovery included the following steps: Swabbing blank determination on ten 100 cm^2 316 L stainless steel coupons was preformed. First TOC grade water was spread on all SS 316 L coupons, subsequently obtain the water sample with swab and analyzed on TOC for blank determination. Then impregnated 150 ng ml^{-1}, 300 ng ml^{-1} and 350 ng ml^{-1} on swabs and poured into 20 ml TOC grade vials containing the same water which was used in blank preparation and vortexed for 10 s and analyzed for TOC for standard readings. For recovery studies we spread (150 ng ml^{-1}, 300 ng ml^{-1} and 350 ng ml^{-1}) of standard solution in an area of 10 × 10 cm on nine coupons. We desorbed a polyester tipped swab in TOC vial containing 20 ml TOC grade water. Using one side of the moistened swab, swab 100 cm^2, moving from left to right and pour into TOC vial containing 20 ml TOC grade water. Using dry swab perform additional swabbing on the same sampling area without desorbing into the water. The sealed TOC vial is vortexed for 10 s and analyzed for TOC. According to the ICH recommendations,[16] precision was considered at two levels, repeatability and intermediate precision. On this account, six-sample replicates were consecutively tested in the same equipment at a concentration of 100% of the regular analytical working value.

4. Evaluation of maximum allowable carry over

The maximum allowable carry over limit of cephradine as potential cross-contaminant was calculated through several methods.[18] The total surface area of the equipment chain in direct contact with the product was accounted for in the calculations. This accounts also for the maximum daily intake of a following product and for its batch size that will be manufactured next with the same equipment. 0.1% approach was calculated by

$$MAC = \frac{DS(\mu g/cm^2)}{IFA}$$

10 ppm approach was calculated by

$$MAC = 10\ x\ S\ (1/A)\ (\mu g/cm^2)$$

Where, MAC is the maximum allowable carry over 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 100 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 on the basis of the assumption that all the products come into contact with all the equipment pieces of the chain.

5. Results and discussion

5.1 Method validation

5.1.1. Accuracy. The recovery value for each concentration was calculated by comparing the blank corrected recovery mean TOC value to the blank corrected impregnated mean TOC value. The mean recovery data (mean ± R.S.D.) for each level were (115.47 ± 2.25%, 104.30 ± 1.53% and 98.10 ± 2.70% respectively, (Table 1).

5.1.2 Linearity. Linearity was determined at ten levels representing from 30 to 600 ng ml^{-1} (10.0% to 200%) A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient, slope and intercept values, which were 0.99986, 56324.18 and −5.6952 respectively. The limits of detection and quantification were 10 and 30 ng ml^{-1} respectively.

5.1.3 Precision

5.1.3.1 Precision repeatability. Repeatability precision was determined by performing swabbing, which involved spiking cephradine on 316 L stainless steel coupons, recovering the

Table 1 Accuracy

S. No	Swabbing Blank (ppb)/A	Impregnated Sample 150 ng ml^{-1} (X)	Impregnated Sample 300 ng ml^{-1} (Y)	Impregnated Sample 450 ng ml^{-1} (Z)	Impregnated Sample 150 ng ml^{-1} - Swabbing Blank (ppb) (X-A)	Impregnated Sample 300 ng ml^{-1} - Swabbing Blank (ppb) (Y-A)	Impregnated Sample 450 ng ml^{-1} - Swabbing Blank (ppb) (*Z*-A)
1	125	375	632	886	250	507	761
2	128	364	641	881	236	513	753
3	121	361	620	861	240	499	740
4	119	384	618	867	265	499	748
5	124	367	647	874	243	523	750
6	129	387	635	876	258	506	747
7	130	374	637	869	244	507	739
8	124	370	645	874	246	521	750
9	118	369	623	894	251	505	776
10	125	381	619	861	256	494	736
Mean	**124**	**373**	**631**	**874**	**249**	**507**	**750**
SD	**4.0**	**8.6**	**11.0**	**10.5**	**8.9**	**9.3**	**11.7**
%RSD	**3.2**	**2.3**	**1.74**	**1.21**	**3.57**	**1.87**	**1.56**

S. No	Cephradine Recovery 150 ng ml^{-1} (B)	Cephradine Recovery 300 ng ml^{-1} (C)	Cephradine Recovery 450 ng ml^{-1} (D)	Cephradine Recovery 150 ng ml^{-1} (B) – Blank mean	Cephradine Recovery 300 ng ml^{-1} (C) – Blank mean	Cephradine Recovery 450 ng ml^{-1} (D) – Blank mean
1	413	653	837	289	529	713
2	405	645	867	281	521	743
3	428	661	875	294	537	751
Mean	**415**	**653**	**859**	**288**	**529**	**735**
%RSD	**2.81**	**1.23**	**2.33**	**2.27**	**1.51**	**2.72**

%Recovery

S. No	(Swabbing blank corrected recovery / Blank corrected impregnated sample Mean) X 100 % Recovery 150 ng ml^{-1}	% Recovery 300 ng ml^{-1}	% Recovery 450 ng ml^{-1}
1	115.6	104.3	95.1
2	112.8	102.7	99.1
3	118.0	105.9	100.1
Mean	**115.47**	**104.30**	**98.10**
%RSD	**2.25**	**1.53**	**2.70**

cephradine with swabs, and desorbing the swabs into TOC grade water. These swabbing samples were then analyzed for TOC. Swabbing was performed with six replicates using the following cephradine concentrations: 150 ng ml^{-1}, 300 ng ml^{-1} and 450 ng ml^{-1}. The precision repeatability was performed in the same manner as in the accuracy study. The data of Table 2 shows that the average results of precision repeatability within 100 $\pm$ 10.0% of test concentrations of 150 ng ml^{-1}, 300 ng ml^{-1} and 450 ng ml^{-1} and R.S.D. was less than 5.0% (Table 2).

5.1.3.2 Precision intermediate. The second analyst carried out intermediate precision on a different day. The swabbing recoveries were performed in the same manner as in the accuracy study. The average results of precision intermediate were within $\pm$ 10.0% of test concentrations of 150 ng ml^{-1}, 300 ng ml^{-1} and 450 ng ml^{-1} and with 92.3 – 106.8% confidence interval, which indicate a good precision.

5.1.4 Robustness. Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, solution stability, pH, flow rate, injection volume, detection wavelength or diluent composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. In this study, only one factor was evaluated which was solution stability. The stability of swab sample taken from SS coupon was evaluated at room temperature, at intervals of 1, 24, and 48 h.[19] The results obtained

Table 2 Precision repeatability

Swabbing Blank Mean (ppb)					
115					
Impregnated Sample 150 ng ml^{-1}	Impregnated Sample 150 ng ml^{-1} – swabbing blank mean	Impregnated Sample 300 ng ml^{-1}	Impregnated Sample 300 ng ml^{-1} – swabbing blank mean	Impregnated Sample 450 ng ml^{-1}	Impregnated Sample 450 ng ml^{-1} – swabbing blank mean
415	300	649	534	890	775
401	286	615	500	902	787
398	283	641	526	867	752
411	296	648	533	856	741
387	272	610	495	892	777
408	293	621	506	859	744
	Mean = 288.33		Mean = 515.67		Mean = 762.67
	%RSD = 3.53		%RSD = 3.37		%RSD = 2.54
Cephradine Recovery 150 ng ml^{-1}	Cephradine Recovery 150 ng ml^{-1} – swabbing blank mean	Cephradine Recovery 300 ng ml^{-1}	Cephradine Recovery 300 ng ml^{-1} – swabbing blank mean	Cephradine Recovery 450 ng ml^{-1}	Cephradine Recovery 450 ng ml^{-1} – swabbing blank mean
435	320	655	540	905	790
425	310	625	510	889	774
405	290	612	497	914	799
425	310	634	519	911	796
401	286	627	512	896	781
409	294	631	516	890	775
	Mean = 301.67		Mean = 515.67		Mean = 785.83
	%RSD = 4.49		%RSD = 2.74		%RSD = 1.36
S.No	Precision result (150 ng ml^{-1})	Precision result (300 ng ml^{-1})	Precision result (450 ng ml^{-1})		
1	106.7%	100.9%	101.9%		
2	108.3%	101.6%	98.3%		
3	102.5%	95.4%	106.3%		
4	104.7%	97.8%	107.4%		
5	105.1%	102.7%	100.5%		
6	100.3	101.6%	104.2%		
Mean	104.6%	100.04%	103.1%		
%RSD	2.75	2.79	3.37		
Lower control limit (LCL)	101.6%	97.1%	99.5%		
Upper control limit (UCL)	107.6%	103.0%	106.8%		

(mean = 107.50%, 110.25%, 91.25% respectively) revealed that samples retained a potency of 100 ± 10% as tested against freshly prepared impregnated standard solution.

5.2. %Recovery from stainless steel, vinyl and glass surfaces

Each plate (S.S. plate, Vinyl and Glass plate) was spread with variable aliquots (150 ng ml^{-1}, 300 ng ml^{-1} and 350 ng/ ml) of standard solution in an area of 10 × 10 cm. Similar procedure, as used in method validation was applied to lift the residues from the surfaces. The % recoveries from each surface showed that the recovery was influenced by the type and the size of surface and not by the level of the drug spiked.

5.3. Establishing limits of cross-contamination on clean equipment

Swab sampling of areas hardest to clean was done from the equipment train used in the manufacturing and residual was found in μg/swab (Table 3). The lowest obtained values were selected as limit of maximum allowable carry over (MAC) for this study.

Table 3 Sample analysis from "hard to clean" areas from the equipment train

Equipment name	Sampling point	Cephradine (μg/ cm^2)		
		Batch 01	Batch 02	Batch 03
Cone blender	Dispensing end	52.01	41.6	21.54
	Side wall	21.21	17.63	14.72
	Outlet mouth wall	12.12	13.09	12.15
Grall mixer	Outlet	6.9	9.80	11.6
	Near gasket	61.68	69.6	38.2
	Blades (chopper)	8.9	68.4	55.7
Fitz-mill	Inside grooves	221.5	324	142
	Sieve bottom	247	296.2	315.4
Encapsulation machine	Inside punch assembly	258.6	149.8	146.8
	Inside dye assembly	44.37	35.6	69.3
	Inside dye	22.29	6.80	60.3
Blister machine	Brush	9.6	2.1	3.8
	Belt	8.4	6.7	5.9

A lowest calculated value of 315 μg cephradine/cm^2 was obtained when the 0.1% dose limit criterion was used for the total equipment chain which was 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.[18]

The lowest calculated value was obtained when 10 ppm acceptance criterion was applied. When less than 10 ppm of cephradine was allowed into the next manufactured product, a limit of 587 μg cephradine/cm^2 was determined as MAC.

5.4. 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. For the current study it was observed that all data obtained lie within 2*s* of the sample mean and well below the MAC (Table 3). This gave the confidence that the manual cleaning procedures tested do provide sufficient removal of the residues from the equipment train.

6. Conclusion

A rapid and reliable TOC method for determination of residues of cephradine on pharmaceutical manufacturing plant equipment has been developed and validated. This assay technique fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, recovery and precision data. It is a non-specific and precise analytical procedure and its quick and rapid analysis allows the analysis of a large number of samples in a short period of time. Therefore, this TOC method can be used for a routine residual analysis. 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.

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