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Short communication

Cleaning validation 1: Development and validation of a chromatographic method for the detection of traces of LpHse detergent

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Abstract

A high performance liquid chromatography (HPLC) method for the detection of traces of LpHse (4-*tert*-amylphenol and 2-phenylphenol) has been developed and validated. The method was shown to be linear in the range from 0.5 to 10.00 ppm in solution. The method was also shown to be accurate with a recovery of up to 95% by area response for amylphenol and up to 94% by area response for phenylphenol from metal surfaces ($4'' \times 4''$ un-polished 304 stainless steel plates) by means of swab material. The reproducibility of the method was determined to be 1.61% by area response and 1.52% by height response for amylphenol and 5.40% by area response and 13.77% by height response for phenylphenol from solutions reported as the pooled relative standard deviation. The developed method was also shown to be rugged by comparisons of different preparations by different analysts. The limit of detection was established to be 0.076 ppm by peak area, 0.079 ppm by peak height for amylphenol and 0.34 ppm by peak area, 0.82 ppm by peak height for phenylphenol from solution, and 1.77 ppb by peak area, 1.23 ppm by peak height for amylphenol and 1.23 ppm by peak area, 1.44 ppm by peak height for phenylphenol from recovery from metal studies. The limit of quantitation was established to be 0.25 ppm by peak area, 0.26 ppm by peak height for amylphenol and 1.14 ppm by peak area, 2.73 ppm by peak height for phenylphenol from solution, and 3.89 ppm by peak area, 4.11 ppm by peak height for amylphenol and 4.11 ppm by peak area, 4.79 ppm by peak height for phenylphenol from recovery from metal plates studies. This method can be employed to determine the presence of LpHse residues in cleaned equipments where the detergent was used.

Keywords: Cleaning validation; Detergent; Method validation

1. Introduction

The validation of cleaning processes in the pharmaceutical industry has been regulated since the inception of the current good manufacturing practices (cGMP's) [1,2]. Reports on cleaning validation of drugs residues by different methods have been published recently [3–7]. However, reports dealing with the detection of cleaning agents after the cleaning process are not commonly found in the literature [8]. The Food and Drug Administration (FDA) expects the manufacturer to evaluate the efficiency of the cleaning process and specified in their guide to inspections validation of cleaning process that no detergent should remain after the cleaning process [9]. The FDA also established that analytical methods had to be developed and validated in order to establish the method's reliability, limits as well as the specificity. The specificity of the method will require that interferences be determined from the matrix. In cleaning validation interferences can come from different sources, sometimes unexpected. Therefore, swabs and solvents used along with the detergent have to be analyzed in order to assess possible interference with the main components of the detergent. Interferences can come from swabbing material, gloves, surface material, active pharmaceutical ingredient, and the environment in general [10].

Commercial detergents present a unique challenge for the analytical chemist in a pharmaceutical environment. Detergent formulations are not patent protected, therefore exact amounts

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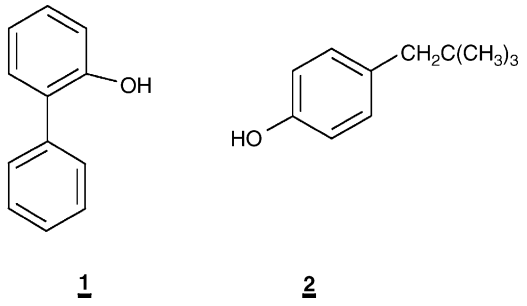

Fig. 1. Chemical structures of phenylphenol (**1**) and amylphenol (**2**).

of "actives" within the detergent are regularly not revealed. Most often these are indicated in ranges that are very wide, and thus hard to predict how much of each per vessel. Therefore, it may be difficult to reproduce the exact concentration of active ingredient of the detergent unless standard solutions are used. In order to generate a calibration curve, the amounts of the components of interest have to be estimated based on general information in the material safety data sheet (MSDS), certificate of analysis from the supplier, or from some unofficial communication from a salesperson. In other instances, the components are commercially available and can be acquired, such as could have been in this study. However, the method required development and validation within a very tight timeframe, which precluded the use of standards for a calibration run. Thus, it was decided to work based on supplier's information as well as on the MSDS data in order to provide a suitable method to support the cleaning validation.

In the case under study, LpHse was used as a detergent and sanitizing agent and thus selected its components to be analyzed as part of the cleaning validation process. LpHse is a detergent and disinfectant used by pharmaceutical manufacturers to remove active from the finished drug product from the manufacturing equipment. The detergent's active ingredients, besides the surfactant, are considered to be the phenols, 2-phenylphenol **1** and 4-*tert*-amylphenol **2**, which act as sanitizing agents (Fig. 1). Conditions for the separation from interferences, identification and quantitation of trace level quantities of LpHse were accomplished. Detailed explanations are found throughout this report and from the data collected.

The present report describes a method developed for the analysis of residual traces of LpHse using HPLC in solution extracts coming from the mechanical removal of the active from metal plates. The validation of the method was performed in accordance with established and accepted practices [11–13].

2. Materials and equipment

2.1. Equipment

The HPLC system consisted of a Shimadzu HPLC system with a SCL-10A system controller, a SIL-10A auto injector, equipped with a SPD-10A UV–vis detector, a LC-10AS pump, a FCV-11AL solvent switcher and a CR-4A chromatopac integrator.

2.2. Materials

The detergent LpHse was obtained from Steris (Mentor, OH, USA). Aqueous solutions were prepared with deionized water produced by a Milli-Q water purification system from Millipore (Bedford, MA, USA). The TEXWIPE TX761 sampling swabs were acquired from the TEXWIPE company (Saddle River NJ, USA). The sampling was made on unpolished metal plates, $4'' \times 4''$ 304 stainless steel. The plastic centrifuge tubes, 50 mL were acquired from Baxter (Deerfield, Illinois, USA). The HPLC methanol, 85% phosphoric acid and HPLC acetonitrile were obtained from fisher (Fair Town, NJ, USA).

2.3. Chromatographic conditions

The column used was a Waters μ-Bondapak, C-18, 10 μm, and 3.9 mm× 150 mm, with a mobile phase composed of acetonitrile: acidified water (pH 2.5) (1:1 v/v), flow rate of 1.0 mL/min and 220 nm wavelength at 0.01 AUFS. The injection volume used was set at 50 μL. The chromatographic experiments were run at room temperature (20 °C).

3. Experimental procedure

3.1. Preparation of mobile phases

HPLC grade acetonitrile: acidified water (pH 2.5) (1:1 v/v) mobile phase was prepared by pippeting 1.00 mL of 85% phosphoric acid to a 1.00 L volumetric flask and diluting to volume with deionized water. The resulting acidified water solution had a pH of 2.5. In a suitable container the acidified water was mixed with HPLC acetonitrile in a 1:1 v/v ratio. The mixture was filtered and degassed. This solution was used as the mobile phase and as the needle wash solution. The solution was also used as the diluent of the sample.

3.2. Preparation of extracting solution

The extracting solution was prepared by mixing HPLC grade methanol and deionized water (7:3 v/v). This solution does not need to be degassed or filtered.

3.3. Preparation of LpHse stock standard solution and the working standard solutions

The LpHse stock standard solution was prepared by pippeting 2.5 mL of the concentrated LpHse detergent to a 1.00 L volumetric flask and diluting the detergent with a mixture of HPLC grade acetonitrile:deionized water (1:1 v/v). From the MSDS data the concentration of amylphenol was found to be approximately 7.4% by weight and for phenylphenol was found to be approximately 7.3% by weight. The specific gravity of un-diluted LpHse is 1.11 g/mL; therefore, the concentration of amylphenol and phenylphenol per mL of LpHse can be estimated to be close to 82 and 81 mg/mL, respectively. In the stock standard solution the resulting concentration of amylphenol was 205.0 and 202.05 ppm for phenylphenol. From the stock solu-

Table 1
LpHse working standard preparation (200 mL final volume)

Aliquot of LpHse stock standard solution (mL)	Theoretical concentration of amylphenol working standard (ppm)	Theoretical concentration of phenylphenol working standard (ppm)
0.50	0.51	0.51
1.00	1.03	1.01
5.00	5.13	5.06
8.00	8.20	8.10
10.00	10.25	10.13

tions an aliquot was taken and diluted to volume with HPLC grade acetonitrile:deionized water (1:1 v/v). The final concentrations of the standards solutions are presented in Table 1.

3.4. Preparation for the recovery of LpHse from metal plates

The recovery from plate solutions were prepared using aliquots from the LpHse stock standard solution and the LpHse 10 ppm standard solution at the following concentrations: for amylphenol the concentrations used were 0.51, 5.13 and 10.25 ppm; for phenylphenol the concentrations were 0.51, 5.06, 10.13 ppm. A volume of 0.50 mL of the LpHse stock standard was spread over a clean and dry $4'' \times 4''$ 304 un-polished stainless steel plate. The procedure was repeated in another plate with a 1.00 mL volume of the LpHse stock standard. Additionally, 1.00 mL of the LpHse 10.00 ppm standard solution was evenly spread over an additional clean plate. The metal plates are allowed to dry under refrigerated conditions (at 4 °C in the refrigerator). Two TEXWIPE TX761 swabs were rinsed with a mixture of MeOH:purified water (7:3 v/v). The wet swabs were passed over the surface of the plate. Afterwards, a second wet swab was passed over the surface of the plate in a manner shown by Fig. 2. Both swabs were transferred to a 50 mL centrifuge tube and 20.0 mL of a mixture of MeOH:purified water (7:3 v/v) was added. The tubes were shaken mechanically for 10 min and each aliquot analyzed by HPLC.

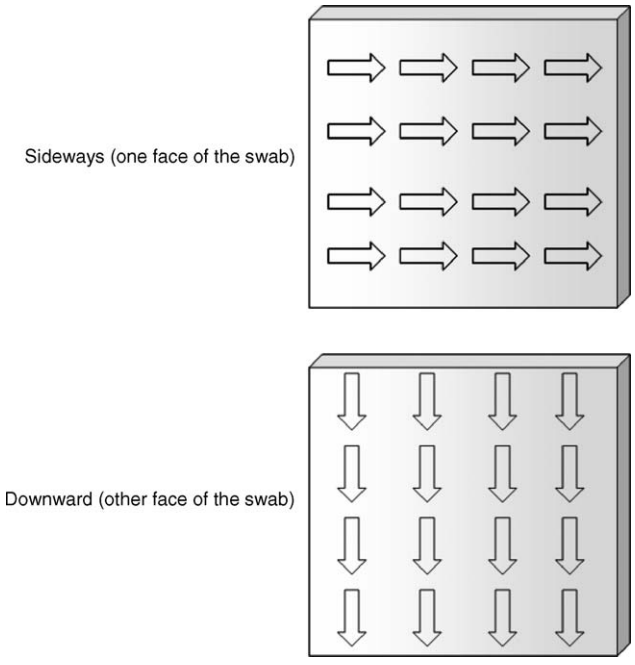

Fig. 2. Swabbing procedure for the recovery experiments from the stainless steel plates. The second swab was passed across the surface in the same manner.

3.5. Statistical calculations

The statistical calculations were carried by means of Statgraphics® software (Windows version 5.0), except for calculations of the grand average Xbar, pooled standard deviation and pooled percentage of relative standard deviation.

4. Results and discussion

4.1. System suitability

The HPLC system suitability was evaluated according to the USP 28 NF 23 for system precision, and tailing factors. The system precision was obtained from the pooled relative standard deviation (co-variance, percentage of RSD_{pooled}) of three sets of replicate injections from different days and preparations. Each replicate set consisted of six consecutive injections. This afforded a percentage of RSD_{pooled} value of 1.07% by area response factor and 1.14% by height response factor for amylphenol and 0.21% by area response factor and 0.33% by height response factor for phenylphenol.

4.2. Reproducibility

The reproducibility of method was determined by using the response factor values obtained for different concentrations using three preparations run on two different days by two different analysts. These were averaged and the pooled standard deviation determined (S_{pooled}). These values were then used to calculate the pooled percentage of RSD that afforded the value of 1.61% by area response factor and 1.52% by height response factor for amylphenol and 5.40% by area response factor and 13.77% by height response factor for phenylphenol. Table 2 presents the chromatographic data for amylphenol and phenylphenol. The data shows that the standard deviations of the measurements have a small value even though the samples were prepared and analyzed by two different analysts (Tables 2 and 3).

4.3. Ruggedness

The ruggedness was shown by two sample comparison of the recovery from plate data for two different preparations by two different analysts, which showed that there is no significant statistical difference between preparations by both area and height responses. A student *T*-test was used to evaluate the ruggedness of the method by comparing the data of two different analysts.

Table 2
Chromatographic data obtained from the HPLC experiments for the standards of amylphenol and phenylphenol

Concentration (ppm)	Average area response ($n=6$)	STD area response ($n=6$)	Average height response ($n=6$)	STD height response ($n=6$)
Amylphenol				
0.51	5920.17	198.15	325.17	5.42
1.03	11676.50	292.88	635.17	13.32
5.13	60445.33	1219.61	3259.67	20.70
8.2	95014.50	1241.13	5114.83	57.57
10.25	118909.67	1269.29	6411.00	98.92
Phenylphenol				
0.51	18882.67	297.47	1356.00	15.09
1.01	37368.00	569.61	2675.67	35.95
5.06	190343.33	3334.78	13475.67	184.55
8.1	292160.50	3776.54	18765.67	13.84
10.13	329351.17	4215.28	18760.00	17.62

In order to compare the two sets of data the following equation was used:

$$x_1 - x_2 = \pm t s_{\text{pooled}} \sqrt{\frac{N_1 + N_2}{N_1 N_2}}$$

The data for the comparison of the two analysts in terms of peak area for all the concentrations of amylphenol and phenylphenol compared favorably and no significant differences of the mean peak areas were found. When the equation is solved for the two different averages, if the difference of the averages is smaller than the calculated value (at the right side of the equation), the null hypothesis is supported and no significant differences of the mean values are established [15]. The analysis was done using the value of t at a 95% confidence level with four degrees of freedom in each calculation (2.78). From the results it can be concluded that the method is rugged enough to allow two different analysts to work on the determination of amylphenol and phenylphenol without significant statistics differences.

Table 3
Data for the T-test of amylphenol and phenylphenol as calculated from the peak area of the analytes by two different analysts

Concentration of amylphenol (ppm)	Difference of the average values (peak area)	T-test calculated from data (peak area)
0.51	112	478
1.03	12	743
5.13	78	2866
8.20	186	3138
10.25	435	3117
Concentration of phenylphenol	**Difference of the average values**	**T-test calculated from data**
0.51	473	474
1.01	627	1152
5.06	1069	16662
8.10	1984	9178
10.13	1796	10400

4.4. Accuracy/recovery

A linear regression of the instrument response area versus experimental concentration was performed to obtain the correlation coefficients (Table 2). These values can be used to judge the linearity of method. The results for the correlation coefficient was $r=0.9998$ ($r^2=99.95\%$) by area response and $r=0.9743$ ($r^2=99.35\%$) by height response for amylphenol and $r=0.9954$ ($r^2=99.08\%$) by area response and $r=0.9743$ ($r^2=99.35\%$) by height response for phenylphenol.

Figs. 3 and 4 present representative chromatograms of the blank and the standard solution having 5.06 ppm of phenylphenol and 5.13 ppm of amylphenol. The accuracy of the method was approximated by a complete procedure involving the mechanical removal of LpHse from a metal plate using swabs. The percentage of recovery was calculated from the equation of the calibration curve considering the peak area of amylphenol and phenylphenol. The samples were tested at three different concentrations: for amylphenol the concentration of the samples were 0.51, 5.13 and 10.25 ppm. The concentrations for phenylphenol were 0.51, 5.06 and 10.13 ppm. The recovery for amylphenol was 65% for 0.51 ppm, 95% for 5.13 ppm and 81% for 10.25 ppm of the substance in the sample. For phenylphenol, the recovery percentage was 39% for the sample containing

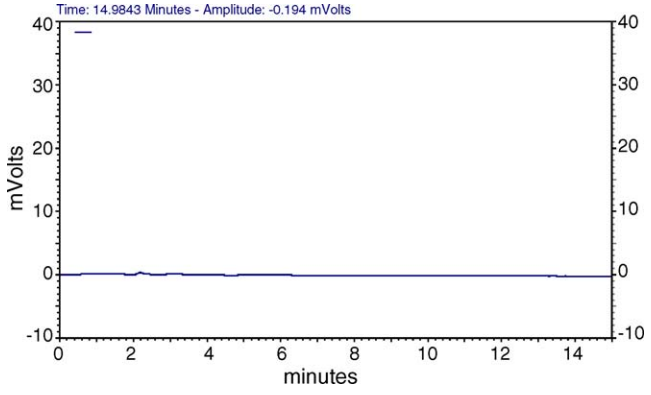

Fig. 3. Representative chromatogram of the blank run having acetonitrile:acidified water (pH 2.5) (1:1 v/v). Sample ran at room temperature, 1 mL/min UV detection at 220 nm.

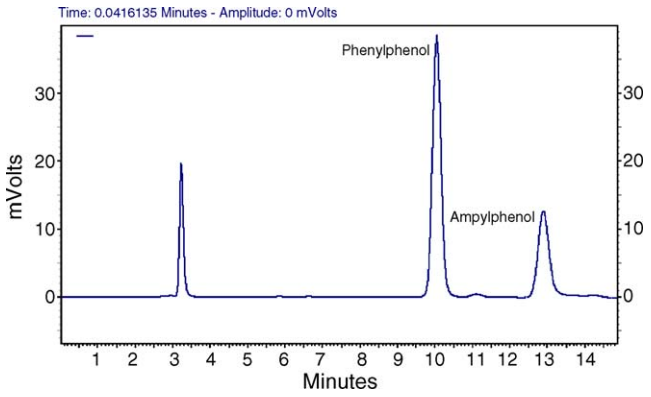

Fig. 4. Representative chromatogram of a standard detergent sample containing 5.06 ppm of phenylphenol and 5.13 ppm of amylphenol. Mobile phase: acetonitrile:acidified water (pH 2.5) (1:1 v/v). Sample ran at room temperature, 1 mL/min UV detection at 220 nm.

0.51 ppm, 94% for the one containing 5.06 and 92% for the one containing 10.13 ppm of the substance.

It is relevant to discuss the phenomenon discovered while performing the recovery experiments. During these experiments it was found that upon drying at room temperature the recovery of the phenols, both the amyl and phenyl were very low, particularly when small concentrations were tested. The material however was still present for the analysis and some of it was recovered. These were then allowed to evaporate at low temperature (2–8 °C). This way, most of the material was collected with the swabbing procedure. Furthermore, a preliminary set of experiments showed that some of the amylphenol was lost upon drying at room temperature while at low temperature the ratio of the two analytes remained. When the samples were dried at room temperature the ratio of the peak areas of phenylphenol:amylphenol was 4.73 on average while the ones dried at 4 °C had an average ratio of the peak areas of phenylphenol:amylphenol of 2.68. From the chromatograms we can speculate that the amylphenol have some evaporation when dried at room temperature. However, this data is preliminary, and more investigation on this area is required to be able to draw any conclusions.

4.5. Sensitivity

4.5.1. Limit of detection (DL)

Using the linearity curve of response area versus experimental concentration the limit of detection was obtained by multiplying by three the ratio of standard error of the intercept to slope of the line. The calculated values afforded a DL of 0.076 ppm by area response and 0.079 ppm by height response for amylphenol solutions and 0.34 ppm by area response and 0.82 ppm by height response for phenylphenol solutions. However, the DL obtained from the recovery from plate linear regression affords a value of 1.17 ppm by area response and 1.23 ppm by height response for amylphenol and 4.11 ppm by area response and 4.79 ppm by height response for phenylphenol.

4.5.2. Limit of quantitation (QL)

The limit of quantitation was obtained in a similar fashion to that of the DL, the ratio of the standard error of the intercept to the slope of the line was multiplied by 10. The calculated values afforded a QL of 0.25 ppm by area response and 0.26 ppm by height response for amylphenol solutions and 1.14 ppm by area response and 2.73 ppm by height response for phenylphenol solutions. However, the QL obtained from the recovery from plate linear regression affords a value of 3.89 ppm by area response and 4.11 ppm by height response for amylphenol and 4.11 ppm by area response and 4.79 ppm by height response for phenylphenol.

5. Conclusion

The method developed for the determination of traces of LpHse is practical and cost effective for cleaning validation processes using this detergent/disinfectant combination. It can be considered valid for the determination traces of the active in swabs material and solutions by area or height response using phenylphenol as the indicator of absence of traces of detergent. It was also found that the method is rugged as it was evaluated with two different analysts and the data obtained by them were statistically similar. Amylphenol and phenylphenol were present in all the analyzed samples, even at small concentrations of the actives. The drying process at low temperature produced more reproducible data since when dried at room temperature the data suggest that some of the amylphenol was evaporated. Amylphenol and phenylphenol have high boiling points (255 and 283 °C, respectively) and it is unlikely that much evaporation would take place. More research is on its way to elucidate whether or not the evaporation of the phenols could affect the analysis. The method can be a model for cleaning validation of detergents and disinfectants in the pharmaceutical industry.

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