IJBPAS, March, 2024, 13(3): 1183-1193

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

QUANTITATIVE DETERMINATION AND SAMPLING OF AMITRYPTYLINE HYDROCHLORIDE RESIDUE FOR CLEANING VALIDATION IN THE PRODUCTION AREA

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Received 10th Feb. 2023; Revised 25th April 2023; Accepted 14th July 2023; Available online 1st March 2024

https://doi.org/10.31032/IJBPAS/2024/13.3.7845

ABSTRACT

A tricyclic antidepressant is amitriptyline hydrochloride. It is converted to nortriptyline, which prevents norepinephrine and serotonin from being reabsorbed. Amitriptyline hydrochloride only undergoes hepatic metabolism in the body. The liver demethylates amitriptyline to nortriptyline, which is the drug's main active metabolite. The proposed work's main goal is to clean and validate equipment used in the production of amitriptyline hydrochloride tablets, such as the coating pan, planetary mixer, and fluid bed drier. The developed RP-HPLC method was used to determine the residue of the selected drug. In terms of accuracy, precision, sensitivity, specificity, and robustness, the developed approach was validated. Amitriptyline hydrochloride residue was determined using the aforementioned described method during cleaning validation. The mobile phase used in this RP-HPLC chromatographic method was acetonitrile: NaH_2PO_4 with H_3PO_4 (60:40) v/v pH 2.5, with a flow rate of 1 ml/min, and the detection wavelength was 247 nm. Spinzarp C_{18} columns (10 cm × 4.6mm i.d. with a particle size of 5µm) were used in the development of this method. The developed method was validated according to ICH guidelines and remains unaffected by small but deliberated changes in experimental conditions.

Keywords: Amitriptyline Hydrochloride; Cleaning Validation; RP-HPLC

INTRODUCTION

One of the most crucial responsibilities in the pharmaceutical sector is cleaning to prevent cross-contamination for subsequent batches produced on the same machinery. Before cleaning validation, analytical methodologies used to identify residuals or pollutants should be verified for the substance or class of chemicals to be analyzed (e.g., API residue, detergent residue) **[1-3]**.

For cleaning control or validation, guidelines suggest using thin layer chromatography (TLC), UV photometric, total organic carbon analysis (TOC), conductivity, gas chromatography (GC), and traditional high-performance liquid chromatography (HPLC). Cleaning validation aims to demonstrate, using a validated analytical approach, that the cleaning operation is effective in removing product residues and excipients, degradation products, cleaning agents, and other potential contaminants. Cross-contamination risk in the manufacturing area can be significantly decreased with this approach **[4-5]**.

Other analytical techniques have also been discussed, such as capillary gas chromatography, over-pressured layer chromatography (OPLC), and micellar electrokinetic chromatography (MEKC). Compared to the aforementioned approaches, Ion Mobility Spectrometry (IMS) and TOC are faster, however, TOC lacks specificity and IMS is typically not offered at pharmaceutical manufacturing plants. Pharmaceutical cleaning verification approaches using liquid chromatography-mass spectrometry (LC-MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) provide the benefit of higher sensitivity, selectivity, and universal applicability even for UV-inactive chemicals. These methods are still uncommon in cleaning control analysis and are more expensive than the other methods discussed above. The most widely used approach for cleaning, control, and validation nowadays is HPLC-UV. The pharmaceutical dosage form is produced at a multiproduct facility called a manufacturing facility. On the basis of the product changeover cleaning that will be used for each piece of equipment, cleaning processes have been developed. Calculated surface areas of each piece of apparatus are added to the reactor's surface area **[6-7]**.

The resulting HPLC method reported here has been validated in accordance with the ICH guidelines on the validation of analytical methods Q2A and Q2B.

Sampling methods for cleaning validation [7]

There are many types of sampling methods

- **Swab sampling method**
- **Rinse sampling method**
- **Coupon Sampling Method**
- **Solvent Sampling Method**
- **Product Sampling Method**
- **Placebo Sampling Method**
- **Swab sampling method (direct surface sampling)**

  This technique involves physically removing any residue that remains on a piece of equipment after cleaning and drying it. To do this, a swab is wetted with a solvent and rubbed over the surface area of the sample. The solvent is extracted into a defined volume and the amount of contamination per swab is calculated using a sensitive analytical technique. The spots must be carefully picked to represent the worst possible locations on the apparatus and the outcome extrapolated to take into account the entire product contact surface area. This formula is used to make a worst-case assessment of potential carryover into the following products. A swab recovery study is performed to determine the ability of the swab to remove the contaminant from the surface sampled.

- **Rinse sampling method:**

  This method is based on the analytical determination of a sample of the last rinsing solvent used in the cleaning procedure. The volume of solvent used for the last rinse must be known to allow for the quantitative determination of contamination. The solvent rinse occurs after cleaning has been completed and is important to ensure appropriate recovery for residues being quantified. This method allows for greater ease of sampling than swabbing but does not cover the entire surface area.

MATERIAL AND METHOD

Reagent and solvent

Acetonitrile, methanol, Sodium-Dihydrogen potassium phosphate, and water (HPLC grade) were purchased from Finer Chem Ltd. Amitriptyline hydrochloride was obtained from Reliance formulation Pvt. Ltd.

Equipment

High-performance liquid chromatographic system (Shimadzu); Software LC solution version 2.53 rotatory injection valve, preparative purification pump LC-20AP, Rheodyne® with a loop of 20L; SPD-M20A photodiode array detector. Fraction collector FRC-10A

Chromatographic condition

ACN: NaH2PO4:H3PO4 (60:40% v/v) and pH 2.5 were combined to create the mobile phase. The mixture was ultrasonically processed for 15 minutes, then passed through a 0.2 m syringe filter. Using isocratic elution as the operating mode, HPLC was used in this work. With a flow

rate of 1.0 mL/min, 40°C column temperature, and a 10 L injection volume, Spinzarp C18 columns (10 cm 4.6mm i.d., with a particle size of 5 m) were employed. It was used to detect at 247 nm. The time for Amitriptyline HCl was around 3.19 minutes.

Method of Swabbing

Cotton that has been treated before was used for swabbing. The operator put on latex gloves to do the swabbing. Tweezers were used to handle the swab. The operator never made contact with the swab.

The swab was dampened with the proper amount of filtered water (approx. 1ml). Here, the term "moistened" refers to a quantity of liquid adequate to completely cover the cotton. The specific piece of machinery or equipment surface was swabbed immediately after the cotton had been soaked. Each time, a surface area of 25 cm^2 was swabbed. The procedure for wiping the surface is outlined in the diagram below. The surface was swabbed in a motion akin to painting. The swab was administered twice, once in a North-South direction and once in an East-West motion (after rotating at 180 °C).

The cleaned swab was vortexed in a cyclo-mixer for 2 minutes after being dipped in 25 ml of filtered water. A verified HPLC method of analysis was used to examine the resultant solution. The sample concentration and the standard concentration were used to calculate the drug concentration. Based on the surface area, the drug concentration for each piece of machinery or equipment was estimated **[8]**.

Establishing cleaning limits

The acceptable drug residual limit must guarantee that subsequent batches produced in the impacted machinery are free from cross-contamination. According to FDA guidance, residual limit determination practices must be reasonable, doable, and verifiable. Technology development is done at Gedeon Richter Plc's hormone prototype plant. The cleaning process cannot be validated; however, it can only be checked while a technology and equipment line is still in the development stage. The possibility of changes to the product line and technology must be considered. This idea enables the setting of restrictions independent of the sequence of production. The analytical techniques' current limits can be used. The patient should not consume more than 0.1% of the recommended therapeutic dose, according to the fundamental tenet of cleansing verification and validation (effective dose). Dose Criteria for the cleaning of equipment calculated the maximum permissible carryover. Using the equation, the maximum permitted carryover (MACO) limit was determined **[9-10]**.

Method validation

The method validation was performed in accordance with the Q2R1 guideline **[11-12]**.

Specificity

For Amitriptyline hydrochloride, the specificity of the established analytical approach was tested. To ensure that no contaminants influenced the results, a placebo solution was placed onto a Spinzarp C_{18} column.

Linearity of response

The method's linearity was established in order to assess how effectively equipment surfaces were cleaned. By rinsing and swabbing, the cleaning procedure was confirmed on a glass plate and stainless steel.

Recovery from Swab

The glass and stainless steel plate were washed and dried. A precise amount of five distinct concentrations (10, 20, 40, 80, 160, and 320 µg/ml) of standard solution were transferred on a stainless steel or glass plate and allowed to gently evaporate.

Accuracy

Three replicates of the determination were performed. By adding three different concentrations of the level at 80%, 100%, and 120% to previously examined samples of amitriptyline HCl (40µg/ml), the recovery studies were carried out in triplicate.

Precision

The glass and stainless steel plate were washed and dried. A precise 40 µg/ml STD solution was transferred to a glass or stainless-steel plate and allowed to gently evaporate. To achieve the final concentration of the solution, which is 4000 ng/ml, SS/glass plate was swabbed with the cotton solution and transferred to a 10 ml volumetric flask.

Intraday precision

Precision during the same day refers to variations in outcomes. Using a standard solution of amitriptyline HCl 4000 ng/ml, the intraday precision was assessed three times on the same day using six replicates.

Interday Precision

The difference in outcomes from day to day is known as inter-day precision. Using a typical Amitriptyline HCl solution containing 4000 ng/ml, the Interday precision was assessed using six replicates, each performed three times on three different days.

The peak area was recorded at 247 nm, and based on the standard peak area, the % RSD was determined.

Limit of Detection

The visualization method was used to determine the LOD. Lower dilutions of the standard medication were prepared using the visualization approach, and the peak area at 247 nm was noted. Amitriptyline HCl, at a concentration of 10 ng, was present in the resultant solution.

Limit of quantification

The visualization method was applied to the LOQ determination. The standard medication was diluted more heavily for the visualization approach, and the peak area at 247 nm was noted. Amitriptyline HCl, at a concentration of 100 ng, was present in the resultant solution.

RESULT AND DISCUSSION

Identification of Amitriptyline HCl using FT-IR spectrometer

Samples were prepared of the pinch of AMY HCl with 20 mg of KBr **(Table 1, Figure 1)**.

METHOD DEVELOPMENT AND CLEANING VALIDATION OF AMITRIPTYLINE HCL BY HPLC METHOD (Figure 2)

Figure 2 shows a Chromatogram of Amitriptyline HCl (10 µg/ml) using the optimized mobile phase of ACN: NaH2PO4 with H3PO4 (29:21) v/v pH 2.5 as the mobile phase.

Figure 3 shows the standard linearity overlay of AMY HCl of (1000-32000 ng/ml) which shows the linear response of AMY HCl.

Figure 4 shows the calibration curve of AMY HCl of 1000-32000 ng/ml. were r^2 was found to be 0.999.

Robustness

Robustness data is shown in table small but deliberate change was done in the experimental parameter. Results show that %RSD is below 2%, the method was robust as per ICH guidelines **(Table 6, 7)**.

Cleaning validation by HPLC method

The coating pan, planetary mixture, and fluid bed dryer were the three main instruments employed in the cleaning validation research. For these three instruments, we must choose the surface and edge side to clean using the rinse-and-swab technique **(Table 8)**.

According to the **Table 8**, all of the instruments that were chosen were cleaned, and samples were obtained using the rinse-and-swab procedure from various parts of the instruments. By using the suggested HPLC procedure, all samples were analyzed. All of the instruments were discovered to have been thoroughly cleaned because the analysis's findings fell within the acceptable range.

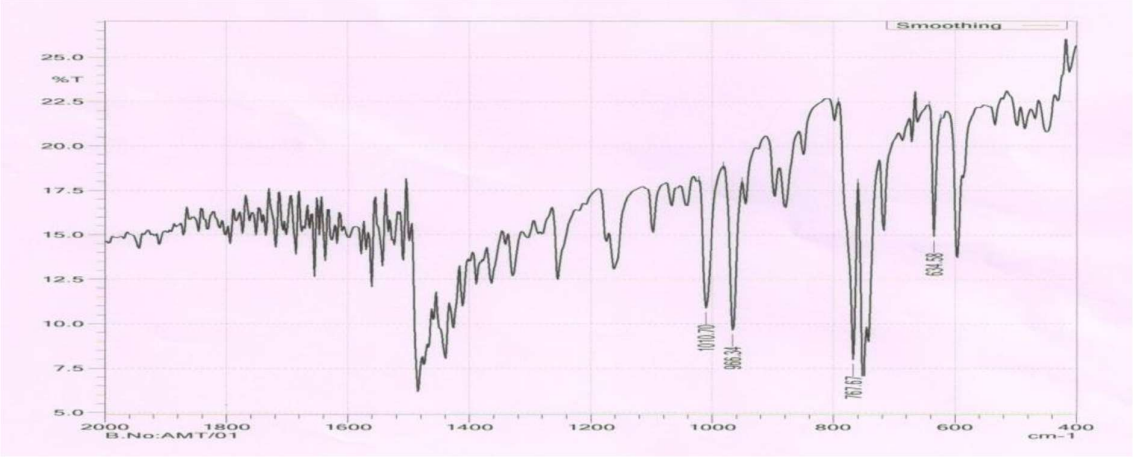

Figure 1: FT-IR spectra of AMY-HCl

Table 1: Interpretation of IR spectra

Sr. No.	IR Range (cm^{-1})	Description
1	1247	Asymmetric C-O-C stretching
2	2934	Aliphatic CH-cyclohexane stretching
3	1467	Aliphatic CH-cyclohexane bending
4	1378	CH-symmetric bending
5	1467	CH-Asymmetric bending
6	850	O-CH3 para-substituted aromatic ring out of plan bending

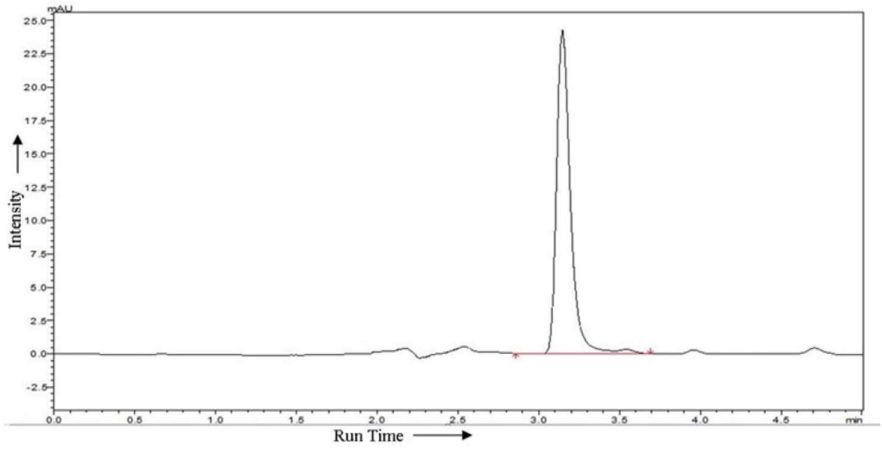

Figure 2: Chromatogram of optimized mobile phase for AMY HCl by HPLC

Table 2: Linearity of AMY HCl by HPLC

Sr. No.	CONC (ng/ml)	Peak area
1	1000	222316
2	2000	461897
3	4000	840270
4	8000	1877889
5	16000	3913096
6	32000	77887779
7	Regression equation	Y=245.3x-56270
8	Correlation coefficient	0.999

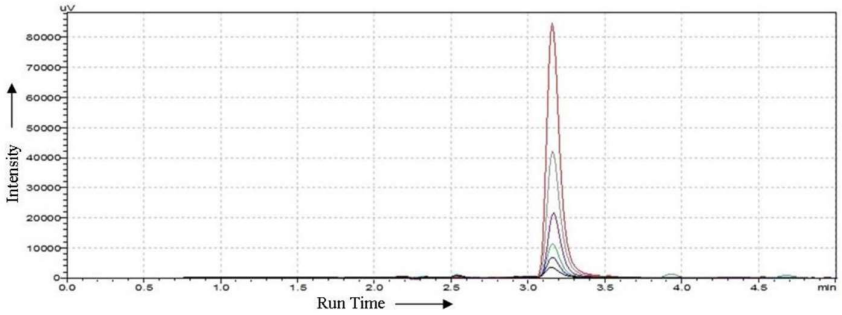

Figure 3: Linearity overlay of AMY HCl (1000-32000 ng/ml)

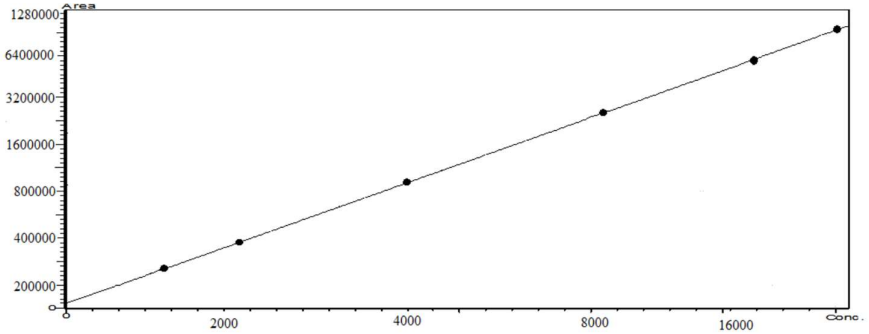

Figure 4: Representative of the standard calibration curve of AMY HCl (1000-32000 ng/ml)

Table 3: linearity of the Amy HCL in Material Substance

Linearity				
Conc range (1000-32000 ng/mL)	Stainless steel Swabbing	Stainless steel Rinsing	Glass plate Swabbing	Glass plate Rinsing
Y-intercept	32805	37378	77723	70411
Slope	234.5	232.93	241.73	238.79
Coefficient	0.998	0.996	0.993	0.997

Table 4: Accuracy of the method for the material substance of product contact surfaces in three concentration levels

Material Substance	Sampling Method	Concentration level		
		80%	100%	120%
Stainless steel	Swab	84.54	84.54	84.54
Glass plate	Swab	87.50	87.50	87.50
Stainless steel	rinsing	86.91	86.91	86.91
Glass plate	Rinsing	89.14	89.14	89.14

Table 5: Precision of the method for the material substance of product contact surfaces in three concentration levels INTRADAY

Material Substance	Sampling Method	Concentration level		
		%RSD		
		80%	100%	120%
Stainless steel	Swab	1.95	1.71	1.90
Glass plate	Swab	1.48	1.66	1.90
Stainless steel	rinsing	1.77	1.46	1.78
Glass plate	Rinsing	1.92	1.56	1.96

INTRADAY

Material Substance	Sampling Method	Concentration level		
		%RSD		
		80%	100%	120%
Stainless steel	Swab	1.88	1.794	1.87
Glass plate	Swab	1.43	1.90	1.66
Stainless steel	rinsing	1.52	1.81	1.81
Glass plate	Rinsing	1.79	1.13	1.86

Table 6: Results of Robustness by HPLC

Change in practical condition	Retention time	Tailing factor	Theoretical plate	Peak area
Mobile phase (31:29)	3.19	1.1	6078	823619
Mobile phase (25:25)	2.19	1.2	6279	804296
Flow rate 1.0mL/min	3.19	1.5	6928	853128
Flow rate 1.5mL/min	2.50	1.3	5329	861420
pH 2.3	3.19	1.4	5496	871428
pH 2 .7	3.19	1.3	6472	862896

Table 7: Summary of the HPLC method

Method	Result			
Drug name	Amitriptyline HCl			
Linearity range (ng/ml)	1000-32000			
	Swab Method (SS)	Rinse method (SS)	Swab method (GS)	Rinse method (GS)
Linearity equation	Y=245.4X-56270	Y=231.3X+ 71514	Y=238.7+ 70411	Y=231.37X+ 71514
Correlation coefficient	0.999	0.997	0.997	0.995
Accuracy (n=3) (% recovery)	84.54-88.32	86.3-93.39	89.61-93.39	89.14-93.54
Precision (%RSD) (n=6)				
Intraday precision (%RSD)	1.79	1.52	1.68	1.81
Interday precision (%RSD)	1.71	1.46	1.50	1.46
LOD (ng/ml)	10			
LOQ (ng/ml)	100			

Table 8: Cleaning validation report by HPLC method

Name of Instrument	Part of Instrument	Sampling technique	Acceptance limit in ppm	Peak area	Result in ppm (based on acceptance limit)
Coating pan	Surface	Rinse	3.91	102197	0.646
	Side of edge			129594	0.757
	Surface	Swab	9.76	121920	0.726
	Side of edge			149674	0.839
Planetary mixture	Surface	Rinse	3.995	249612	1.246
	Side of edge			259679	1.288
	Surface	Swab	8.18	269078	1.326
	Side of edge			391984	1.877
Fluid bed dryer	Surface	Rinse	4.97	409614	1.899
	Side of edge			497897	2.559
	Dryer Surface	Swab	10.32	4059677	2.130
	Side of edge			501496	2.273

SUMMARY AND CONCLUSION

Amitriptyline Hydrochloride is an antidepressant drug approved in May 1983 and marketed by Elavilas a brand name AstraZeneca Pharmaceuticals.

HPLC method has been used for the quantification of amitriptyline hydrochloride and validated in terms of linearity, precision, accuracy, LOD, LOQ, specificity, and robustness. ACN: NaH_2PO_4 with H_3PO_4 (60:40) v/v pH 2.5 was a mobile phase. The linearity was found to be Y=245.3x 56270, r^2 = 0.99 9 and Y=231.3x+71514, r^2 =0.997 three batches consecutively respectively. Similarly, the linearity equation for the swab and rinse method on glass was found

to be Y = 7x+7041 1, r^2 =0.997 and Y=231.37x+71514, r^2 =0.997 for three consecutively batches respectively.

The % recovery for the swab and rinse method on the ss plate was found to be 88.32-85.74 and 86.91-85.74. Similarly, % recovery for a swab and rinse on a glass plate was found to be 87.50-93.36 and 89.14-93.54 respectively.

The % RSD of intraday and Interday precision for a swab and rinse on the ss plate was found to be 1.79-1.71 and 1.46-1.52 respectively. Similarly, % RSD of intraday and Interday precision for a swab and rinse on a glass plate was found to be 1.50-1.68 and 1.46-1.81 respectively.

The residual limit of different production area instruments was found to be in the cleaning validation method for coating pan in swab and rinse method on surface and side and edge 0.72641 0.839 ppm and 0.7377 ppm respectively. For the planetary mixture and fluid bed dryer, the residual limit was 1.326 1.877 ppm, 1.246 1.273 ppm, and 1.899 2.259 ppm respectively.

Acknowledgment

We would like to thank the Department of Pharmaceutical Sciences at Saurashtra University for providing the facility needed to conduct the current research. We would also like to thank the Department of Pharmaceutical Sciences at Saurashtra University in Rajkot.

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