

# Development and validation of a UPLC method for the determination of duloxetine hydrochloride residues on pharmaceutical manufacturing equipment surfaces

## Abstract

**Background:** In pharmaceutical industries, it is very important to remove drug residues from the equipment and areas used. The cleaning procedure must be validated, so special attention must be devoted to the methods used for analysis of trace amounts of drugs. A rapid, sensitive, and specific reverse phase ultra-performance liquid chromatographic (UPLC) method was developed for the quantitative determination of duloxetine in cleaning validation swab samples. **Material and Methods:** The method was validated using an Acquity UPLC™ HSS T3 (100 × 2.1 mm<sup>2</sup>) 1.8 μm column with a isocratic mobile phase containing a mixture of 0.01 M potassium dihydrogen orthophosphate, pH adjusted to 3.0 with orthophosphoric acid and acetonitrile (60:40 v/v). The flow rate of the mobile phase was 0.4 ml/min with a column temperature of 40°C and detection wavelength at 230 nm. Cotton swabs, moisten with extraction solution (90% methanol and 10% water), were used to remove any residue of drug from stainless steel, glass and silica surfaces, and give recoveries >80% at four concentration levels. **Results:** The precision of the results, reported as the relative standard deviation, were below 1.5%. The calibration curve was linear over a concentration range from 0.02 to 5.0 μg/ml with a correlation coefficient of 0.999. The detection limit and quantitation limit were 0.006 and 0.02 μg/ml, respectively. The method was validated over a concentration range of 0.05–5.0 μg/ml. **Conclusion:** The developed method was validated with respect to specificity, linearity, limit of detection and quantification, accuracy, precision, and robustness.

**Key words:** Cleaning validation, duloxetine, residues, swab analysis, UPLC-UV

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## INTRODUCTION

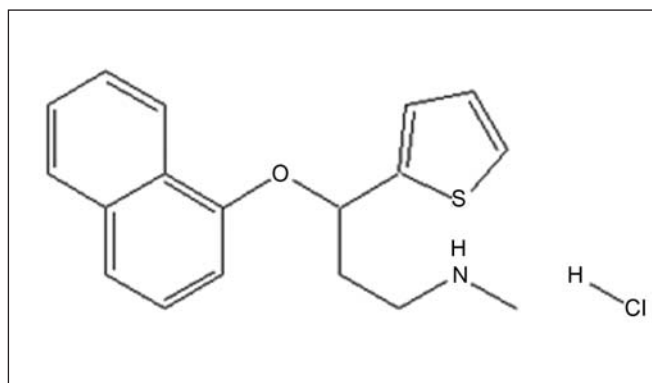
In pharmaceutical manufacturing industries, it is well established that equipments and production areas must be thoroughly cleaned after each manufacturing process, and regulatory authorities recommend validation of the procedure used. Cleaning validation is the process of ensuring the cleaning procedure which effectively removes the residues from the manufacturing equipment and facilities below a predetermined level. This is necessary not only to ensure the quality of the next batch of different products but also to prevent cross-contamination; it is also a World Health Organisation good manufacturing practice (GMP) requirement. Cleaning validation consists of two separate activities: development and validation of the cleaning procedure used to remove the drug from the manufacturing equipment surfaces and development and validation of the methods used to quantify the residues on the surfaces of manufacturing equipments.<sup>[1,2]</sup>

The sampling, therefore a very important parameter, since the conclusion of the cleaning procedure is based on the sample results. According to the FDA guide,<sup>[2]</sup> two different methods of sampling are generally admitted for performing a cleaning control: the direct surface sampling, using the swabbing technique and the indirect sampling based on the analysis of solutions used for rinsing the equipment.

The acceptance limit for residues in the equipment is not established in the current regulations. According to the FDA, the limit should be based on logical criteria, involving the risk associated with residues of determined products. Calculation of an acceptable residual limit, maximum allowable carryover (MAC), for active products in the production equipment should be based on therapeutic doses, toxicity, and a general limit (10 ppm). Several mathematical formulas were proposed to establish the acceptable residual limit.<sup>[3-5]</sup>

Duloxetine hydrochloride is a selective serotonin and norepinephrine reuptake inhibitor for oral administration, used for depressive disorders. Its chemical designation is (+)-(S)-N-methyl-β-(1-naphthoxy)2-thiophenepropylamine hydrochloride [Figure 1]. Its empirical formula is  $C_{18}H_{19}NOS \cdot HCl$ , which corresponds to a molecular weight of 333.38. Its solid oral dosage form is available as a capsule which contains enteric-coated pellets of 22.4 mg, 33.7 mg, and 67.3 mg of duloxetine hydrochloride equivalent to 20 mg, 30 mg, and 60 mg of duloxetine, respectively.<sup>[6]</sup>

Several LC methods have been published for determination of duloxetine in pharmaceutical preparation<sup>[7-11]</sup> and human plasma.<sup>[12,13]</sup> Reported HPLC methods are not enough sensitive to quantitate the trace level amount of duloxetine HCl present in swab samples. A literature survey revealed that no validated cleaning method for duloxetine is to be found. Due to their high sensitivity and selectivity, analytical methods such as liquid chromatography were previously used for the determination of residues to control cleaning procedures.<sup>[14,15]</sup> In liquid chromatography, the analysis time can be reduced by using small columns packed with sub-2 μm particles. In addition, with sub-2 μm particles, due to the higher efficiency and smaller retention volume, sensitivity is also improved, compared to conventional HPLC. A dedicated low dispersion system for ultra-high



**Figure 1:** Structure of duloxetine hydrochloride

pressure separation (UPLC) with the particle size of the stationary phase reduced down to 1.7 μm, small dwell and extra column volume is able to work up to 1000 bar (15,000 psi). In such a way, the analysis time could be reduced down to 2–3 min.<sup>[16]</sup>

The aim of this study was to demonstrate the applicability of UPLC to these purposes by developing and validating an UPLC/UV method to determine the residue duloxetine in cleaning control samples. Hence, we have developed a RP-UPLC method for the estimation of trace level residues of duloxetine on swab collected from manufacturing surfaces after production of duloxetine capsules and cleaning of the equipment. The developed analytical method was validated with respect to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and quantification (LOQ). The stability of duloxetine samples was also studied. These studies were performed in accordance with established ICH guidelines.

## MATERIALS AND METHODS

### Chemicals and reagents

The certified duloxetine hydrochloride, the working standard was supplied by Dr. Reddy's Laboratories limited, Hyderabad, India. The HPLC grade acetonitrile and analytical grade  $KH_2PO_4$  and ortho-phosphoric acid were purchased from Merck, Mumbai, India. High purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA). Swabs for sampling were purchased from ITW Texwipe (Philippines).

### Apparatus

The chromatography analysis was performed using a Waters Acquity™ UPLC separation module (Waters Corporation, Milford, USA) equipped with a UV/visible detector, binary solvent manager and auto sampler system. The output signal was monitored and processed using Empower 2 software. The pH of the solutions was measured by a pH meter (Mettler-Toledo, Switzerland). In the sample preparation, an ultrasonic instrument was used for sonication.

### Chromatographic conditions

The method was developed using an Acquity UPLC™ HSS T3 (100 × 2.1 mm<sup>2</sup>) 1.8 μm column with an isocratic mobile phase containing a mixture of 0.01 M potassium dihydrogen ortho-phosphate, pH adjusted to 3.0 with ortho-phosphoric acid and acetonitrile

(60:40 v/v). The mobile phase was filtered through nylon 0.22  $\mu\text{m}$  membrane filters and degassed. The flow rate of the mobile phase was 0.4 mL/min. The column temperature was maintained at 40 °C and the eluted compounds were monitored at the wavelength of 230 nm. The sample injection volume was 5  $\mu\text{L}$ .

### Standard solution preparation

Milli-Q water and methanol in the ratio of 10:90 v/v was used as diluent. A stock solution containing 0.56 mg/mL duloxetine was prepared by an dissolving appropriate amount of drug in diluent. The final concentration of solution was 0.1  $\mu\text{g}/\text{mL}$  of duloxetine. Appropriate dilutions were made with diluent to obtain solution containing 0.5, 1.0, 5.0, and 50  $\mu\text{g}/\text{mL}$ .

### Sample preparation (extraction procedure)

The selected surfaces (25 × 25  $\text{cm}^2$ ) of stainless steel, previously cleaned and dried, were sprayed with 1000  $\mu\text{L}$  of standard solution, for the positive swab control at all concentration levels and the solvent was allowed to evaporate. The total surface were successively wiped first in horizontal and secondly in a vertical way, starting from outside toward the center, with one or two swabs moistened with extraction solution (water-methanol 10:90, v/v) to remove the residue from the surface. The swabs were placed in the 25 mL screw-cap test tubes containing 10 mL extraction solution. The tubes were placed in an ultrasonic bath for 15 min, and the solutions were analysed by UPLC. Rinse-sampling was performed with extraction solvent. The volume of the rinsing liquid for the sampling point was 10 mL for 625  $\text{cm}^2$  surface.

## RESULTS AND DISCUSSION

### Establishing cleaning limits

The acceptable limit for the drug residue must ensure the absence of cross contamination for subsequent batches manufactured in the affected equipment.<sup>[17]</sup> FDA's guidance for determining residue limits requires a logical, practical, achievable and verifiable determination practice.<sup>[2]</sup>

The basic principle of cleaning verification/validation is that the patient should not take more than 0.1% of the standard therapeutic dose (effective dose). The calculation formula is based on the dosage criteria.<sup>[3,4]</sup>

$$\text{MAC} = \frac{\text{STD}}{\text{SF}} * \left[ \frac{\text{SBS}}{\text{LWSD}} \right]$$

MAC is the maximum allowable carryover, STD is the minimal daily dose (active weight) of previous product, SF is a safety factor (1000), SBS is the smallest batch size of the subsequent product, and LWSD is the maximum daily dose (product weight) of the following product.

An additional criterion is the 10 ppm (part per million) limit.<sup>[5]</sup> According to this criterion, not more than 10 ppm of the previously manufactured product is allowed to appear in the subsequent product. If the value, which is obtained from the calculation based on the dosage criterion, is greater than 10 ppm, then the 10 ppm criterion is applicable. The acceptable limit for residues (LA) is expressed in  $\mu\text{g}/\text{dm}^2$ .

$$\text{LA} = \frac{\text{MAC} * A * R}{\text{TA}}$$

LA is the acceptance limit, A is the sampling area, R is the recovery of the sampling method, and TA is the total production line area.

### Method development and optimization

The main objective in this study has been to develop an UPLC method using isocratic conditions for the analysis of low quantities of duloxetine, trying to get a high peak in a short time. We selected 230 nm for the analysis because the drug has sufficient absorption and low quantities of duloxetine may be detected correctly. Furthermore, the calibration curves obtained at 230 nm show good linearity. The mobile phase very often used is the mixture of phosphate buffer and acetonitrile in different proportions. The run time was too long with the higher pH (above pH 4.0) and higher proportion of the buffer in the mobile phase. To solve this problem, several mobile phases were tested, varying their composition and pH, to obtain the chromatographic separation. The proposed mobile phase composed by 0.01 M potassium dihydrogen ortho-phosphate, pH adjusted to 3.0 with ortho-phosphoric acid and acetonitrile (60:40 v/v) gave best resolution and sensitivity with a very shorter run time. An Acquity UPLC™ HSS T3 (100 × 2.1  $\text{mm}^2$ ) 1.8  $\mu\text{m}$  column was selected over an Acquity UPLC™ BEH C18 (100 × 2.1  $\text{mm}^2$ ) 1.7  $\mu\text{m}$  column, to achieve good peak shape and symmetry. The injection volume was varied between 2 and 10  $\mu\text{L}$ , finally 5  $\mu\text{L}$  was chosen, because bigger volumes imply wider peaks without much enhancement of the signal-to-noise ratio. The flow rate of the mobile phase was kept 0.4 mL/min and the column temperature was maintained at 40 °C.

### Validation of the method

The proposed method was validated as per ICH guidelines [18]. The following validation characteristics were addressed: specificity, accuracy, precision, limit of detection and quantification, linearity, range, and robustness.

#### System suitability

The system suitability test was used to ensure that the UPLC system and procedures are adequate for the analysis performed. Parameters of this test were column efficiency (number of theoretical plates), asymmetry of chromatographic peak, and reproducibility as RSD of peak area of six injections of standard solution. During performing the system suitability test, in all cases relative standard deviation (RSD) of the peak areas was  $\leq 2.0\%$ , the number of theoretical plates per column was 3000, and the USP tailing factor was  $\leq 2.0$ . The results are summarized in Table 1.

#### Specificity

The ability of this method to separate and accurately measure the peak of interest indicates the specificity of the method. The specificity of the method was checked by injecting duloxetine standard, duloxetine sample, the background control sample, and the negative swab control. There is no interference from the extracted blank swab, and the extraction solvent at the retention time of analyte peak [Figure 2].

#### Linearity

Linearity of the method was studied by analyzing standard solutions at eight different concentration levels ranging from 0.021 to 10.2  $\mu\text{g/mL}$ . The calibration curve was constructed by plotting the response area against the corresponding concentration injected, using the least square method. The calibration curve values of slope, intercept, and correlation coefficient for duloxetine are 84655.57, -2436.74 and 0.9999, respectively. The high value of the correlation coefficient indicated good linearity.

#### Limits of detection and quantification

The LOD and LOQ were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of analyte with known concentrations. The precision study was also carried out at the LOD and LOQ levels by injecting six replicates of duloxetine preparation. Calculated the %RSD of the peak area and found  $<3.3\%$  at the LOQ concentration and  $<13.1\%$  at the LOD concentration [Figure 3].

#### Precision

The precision of the chromatographic method,

reported as RSD, was estimated by measuring repeatability and time-dependent intermediate precision on six replicate injections at four different concentrations (0.05, 0.11, 1.04, and 5.19  $\mu\text{g/mL}$ ). The % RSD values presented in Table 2 were  $< 5.7\%$  and illustrated the good precision of the analytical method.

#### Accuracy

Accuracy of the procedure was assessed by comparing the analyte amount determined *versus* the known amount spiked at four different concentration levels (0.05, 0.11, 1.04, and 5.19  $\mu\text{g/mL}$ ) with three replicates for each concentration. The percentage recovery for duloxetine was calculated [Table 3].

#### Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and system suitability parameters for duloxetine HCl standard were recorded. The variables evaluated in the study were pH of the mobile phase buffer (0.2), column temperature ( $\pm 5^\circ\text{C}$ ), flow rate ( $\pm 0.04$  ml/min), wavelength ( $\pm 3$  nm), and % organic in the mobile phase ( $\pm 10\%$ ). In all these deliberate varied chromatographic conditions, system suitability parameters meet the acceptance criteria and RSD of

**Table 1: System suitability test results**

Parameters	Specification	Observed values
Retention time (mean $\pm$ % RSD, n = 6)	$\geq 1.65$ and $\leq 2.15$ min $\pm 1.0$	1.899 $\pm$ 0.1
USP tailing	$\leq 2.0$	1.3
No. of theoretical plates	$\geq 3000$	5134
Area (%RSD, n = 6)	$\leq 5.0\%$	0.5

**Table 2: Results of the precision study**

Spiked level ( $\mu\text{g/mL}$ )	% RSD (n = 6)	
	Precision	Intermediate precision
0.05	4.5	2.9
0.11	3.2	5.4
1.04	5.7	2.6
5.19	2.1	3.8

**Table 3: Results of the recovery study**

Sample	Spiked level ( $\mu\text{g/mL}$ )	Mean % recovery (n = 3)	% RSD (n = 3)
SS plates	0.05	90.4	3.7
	0.11	89.1	2.9
	1.04	86.5	5.2
	5.19	87.8	2.3
Swabs	0.05	98.3	0.6
	1.04	97.5	0.2
	5.19	98.1	0.8

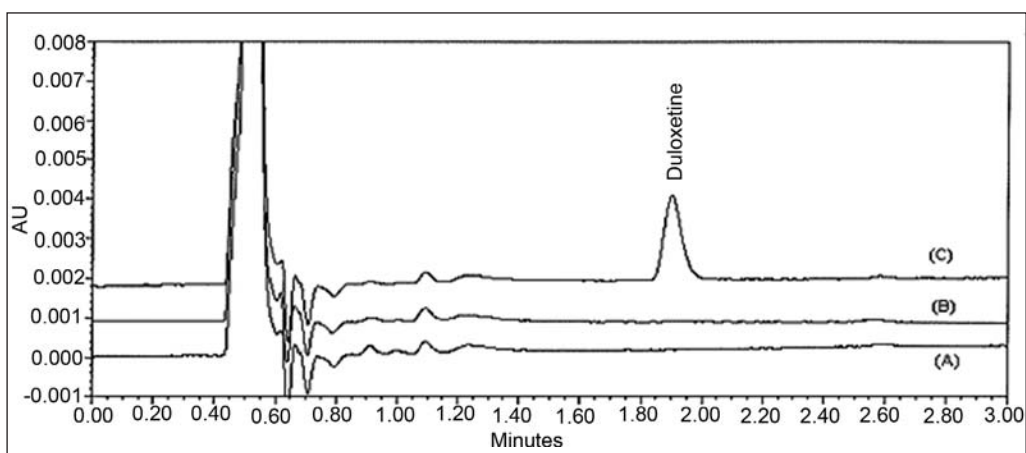


Figure 2: Overlay chromatograms of (A) extraction solvent, (B) extracted blank swab, and (C) active compound spiked at 0.11 µg/mL level

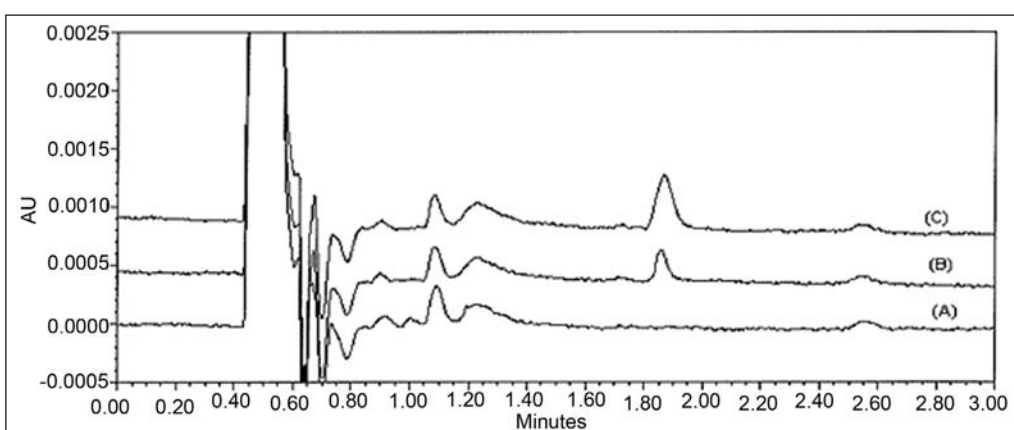


Figure 3: Overlay chromatograms of (A) extracted blank swab, (B) LOD and (C) LOQ level samples

Table 4: Robustness results of the UPLC method

Variation in chromatographic conditions	Observed system suitability parameters			
	Retention time <sup>a</sup> , $\geq 1.65$ and $\leq 2.15$ min $\pm 1.0$	USP tailing, $< 2.0$	No. of theoretical plates $\geq 3000$	% RSD, $\leq 5.0$ (n = 6)
Column temperature 35°C	1.944 $\pm$ 0.1	1.2	5034	0.8
Column temperature 45°C	1.789 $\pm$ 0.1	1.2	4521	0.6
Flow rate, 0.36 mL/min	2.104 $\pm$ 0.1	1.2	4554	1.0
Flow rate, 0.44 mL/min	1.676 $\pm$ 0.1	1.2	4775	0.9
Acetonitrile 90%	2.118 $\pm$ 0.0	1.3	4638	1.1
Acetonitrile 110%	1.712 $\pm$ 0.1	1.2	4593	0.8
Wavelength 227 nm	1.901 $\pm$ 0.2	1.2	5164	0.2
Wavelength 333 nm	1.900 $\pm$ 0.2	1.2	5088	1.2
Mobile phase buffer pH 2.8	1.841 $\pm$ 0.0	1.2	4803	1.2
Mobile phase buffer pH 3.2	1.955 $\pm$ 0.1	1.3	4682	1.5

<sup>a</sup>Mean  $\pm$  %RSD, n = 5.

the peak areas was found to be  $<2.0\%$ , the number of theoretical plates per column was  $>3000$  and the USP tailing factor was  $<2.0$  [Table 4].

#### Solution stability

The stability of the duloxetine in the swab matrix and standard solution was tested. The spiked sample and standard solution were stored at ambient temperature

for 4 days. All the samples were injected into the UPLC system after 1, 2, and 4 days against freshly prepared standard solution. Sample and standard solution were stable up to 4 days. No changes in the chromatography of the stored samples were found, and no additional peak was registered when compared with the chromatograms of the freshly prepared samples.

## CONCLUSIONS

A new sensitive UPLC method has been developed for the simultaneous determination of duloxetine residues on the pharmaceutical manufacturing surface to control the efficiency of the equipment cleaning. The method was validated in accordance with ICH guidelines and found to be specific, precise, accurate, linear, robust, and rugged. Hence, the method can be used as part of a cleaning validation program in the pharmaceutical manufacture of duloxetine.

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