HPLC-DAD Estimation of Nebumetone to Demonstrate Cleaning Validation on Stainless Steel Surfaces of the Production lane

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Abstract: The HPLC-DAD method was very rapid, sensitive and precise which developed for the estimation of Nebumetone to Demonstrate Cleaning Validation on Stainless Steel Surfaces of the Production lane. The Separation of the drug was achieved on a Phenomenex Luna C_{18} (25 cm×5 µm×4.6 mm i.d.) column by using a mobile phase in the ratio consisting of a mixture of methanol:acetonitrile:water (55:30:15, v/v/v). The flow rate was 1 ml/min and the detection wave length 230 nm. The linearity was found in the range of 0.1-4.56 µg/ml with a correlation coefficient of 0.996. The proposed method was validated for its sensitivity, linearity, accuracy and precision. This method was employed for routine quality control analysis of trospium chloride in tablet dosage forms.

Keywords: Nebumetone, Swab-wipes, sampling protocol, HPLC-DAD.

I. Introduction

The part of the Cleaning validation plays an important analytical challenge in pharmaceutical industries. It stems primarily as a part of mandatory for good manufacturing procedure due to the contamination potential. Secondly, for quantitative estimation of residues over the surface of manufacturing equipment after cleaning procedure it requires development of selective and sensitive methods. In the manufacturing lane it involves identification of numerous sampling points to demonstrate complete removal of residues. Current regulatory norms do not establish acceptance limits for residues, but let an analyst decide it on the basis of logical criteria such as risk associated with the quality or safety of finished product.

Generally the limit for maximum accepted residue of active ingredient (maximum allowable carryover, MACO) is based on mathematical formulae, therapeutic doses and toxicological profile, which is kept at a general limit of 10 µg/mL . Several approaches to express acceptance limits have been proposed in the published scientific work. One approach is to compare visual limit of detection (VLOD) with pharmacology based criteria, where not more than 1/1000th of the therapeutic dose of active component should be carried over to the next batch as residue; lower of the two is considered as the residual acceptance criterion. Another approach involves estimating the total amount of allowable residue present on production line, which is termed as residual acceptance level (RAL). Further, the concentration of residue present per unit equipment surface area may be computed, which is termed as specific residual cleaning level (SRCL) or limit per surface area (LSA).

Nabumetone (NAB) is chemically 4-(6-methoxy-2-naphthalenyl)-2-butanone; it is an ester prodrug of a new generation of effective and orally active angiotensin-II receptor antagonist. It blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin-II, one of the most important regulators of blood pressure. The determination of NAB from

tablet formulation has been carried out by high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC) and spectrophotometer, alone or in combination. Several analytical methods have been reported for their determination alone or in combination with other drugs in different dosage forms, biological fluids and urine using different analytical techniques . Available literature revealed that no method related to residual determination of NAB was reported so far; hence it was found worthwhile to determine LSA of NAB and to carry out the development and validation of the method in order to ensure trace level estimation of residues and to demonstrate efficiency of the cleaning procedure.

Experimental Reagent and Chemicals

NAB reference standard (USP) was obtained from IPCA Labs, Ratlam, Madhya Pradesh, India, as a gratis sample. Nilitis (NAB, 500 mg) tablets were procured from the manufacturer. HPLC grade water was prepared by taking reverse osmosis water and passing it through a Milli-Q System (Millipore, Milford, USA). Alpha Swab polyester on a propylene handle-TX714A (ITW Tex wipe, USA) was used for extraction recovery sampling. HPLC grade acetonitrile and methanol were obtained from Merck, Germany. All other chemicals used were of analytical grade.

Chromatographic system and conditions

The LC system consisted of a (Shimadzu LC 10AT VP) gradient pump with a universal loop injector (Rheodyne 7725i) of 20 µL injection capacity, a photodiode array detector (PDA), SPD-10 AVP and Phenomenex Luna C_{18} (25 cm×5 µm×4.6 mm i.d.) column at 1.0 mL/min flow rate, using 20 µL injection volume controlled by a PC work station equipped with the software CLASS-VP (software M-10, version 1.6; Shimadzu. Tokyo, Japan). Column temperature was ambient. The mobile phase consisted of a mixture of methanol:acetonitrile:water (55:30:15, v/v/v). The mobile phase solution was filtered through a 0.45 µm membrane filter (Millipore) and degassed prior to use. The extraction solution consisted of 60 mL mobile phase solution, 20 mL methanol and 20 mL water (50:20:30, v/v/v). All chromatographic experiments were performed in the isocratic mode. UV detection was performed at 230 nm. The method was validated as per ICH guidelines. The statistical analysis was performed using Microsoft Excel 2007.

Standard solution preparation

The stock solution of standard was prepared by accurately weighing NAB reference standard and transferring to a 50 mL volumetric flask. 20 mL of methanol was added and the content of flask was sonicated for 30 min. The solution was appropriately diluted with the mobile phase to get the final concentration of 0.020 mg/mL. A series of calibration standards were prepared by transferring appropriate aliquots of standard NAB solutions to separate 100 mL volumetric flasks to get dilutions.

Sample solution preparation

10 cm×10 cm of a stainless steel surface, appropriately cleaned and dried, was sprayed with 250 µL of standard stock solution for the positive swab control at all concentration levels, and the solvent was allowed to evaporate. The surface was wiped using a wet cotton swab, soaked with extraction solution (mobile phase:methanol:water; 60:20:20, v/v/v). The swab was squeezed into the swab tube as per the procedure mentioned below. The background control sample was prepared from the extraction solvent. The negative swab control

was prepared similarly. Care was taken to avoid contact of swab with the test surface. Subsequently, the tubes were placed in an ultrasonic bath for 15 min and the solutions were analyzed by HPLC-DAD.

Swab wipes sampling protocol

Rinse and swab are two sampling methods available to demonstrate cleaning validation. The swab technique is a technique preferred by United States Food and Drug Administration. The swabbing process is a subjective manual process that involves physical interaction between the swab and the surface, and thus may vary from operator to operator. So, a standardized motion protocol is required to establish reproducible recoveries. A patch of $4\times4in^2$. swab was immersed in the extraction solution and folded diagonally. The excess solution was squeezed to avoid unnecessary dilution of the drug. The folded swab was kept between the thumb and second finger, so that necessary force may be applied over the surface through first finger. The surface was wiped horizontally, starting from outside towards the center. The fresh surface was exposed and repeatedly wiped to extract the maximum residue. Finally the swab was secured in a closed and labeled container for estimation.

Acceptance limit calculation

Cleaning validation of production lane is one of the most critically controlled tasks. Visual as well as analytical observations help to achieve the goal. Considering SRCL, VLOD, MACO and stainless steel surface area of 10 cm×10 cm, the calculated limit per surface area (LSA) was decided as 2 µg swab per 100 cm^2.

Optimization of chromatographic conditions

Best chromatographic conditions were achieved by optimizing the wavelength for detection, mobile phase composition and flow rate. The mobile phase consisted of a mixture of 55 mL methanol, 30 mL acetonitrile, and 15 mL water (55:30:15, v/v/v). Chromatographic conditions were optimized to achieve appropriate plate numbers, peak symmetry, resolution and tailing factor. The calibration curve showed good linearity for lower concentrations, required for trace level estimations at 230 nm.

Optimization of sample treatment

Cotton swabs were spiked with different quantities of drug and placed into tubes. The optimum conditions were achieved with mobile phase:methanol:water (60:20:20, v/v/v) as the extracting solvent and sonification time of 15 min.

Validation of the method

The main objective of this study was to develop an HPLC-DAD method for estimation of residues collected by swabs, without interference of impurities originating from the swabs, plates and extraction media. The method was validated for linearity, precision, limit of detection (LOD), limit of quantification (LOQ), accuracy, selectivity, and stability of analyte .

System suitability

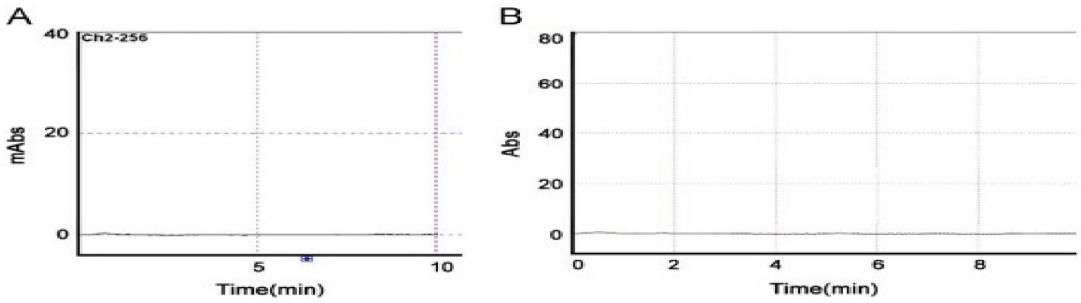
The average number of theoretical plates per column was >3400, the USP tailing factor <1.2 and the resolution >2.0. Relative standard deviation (RSD) of the peak areas was <2.0%.

Specificity

The specificity of the method was checked by using standard, samples, the background control sample, the negative swab control, and a swabbed un-

spiked stainless steel plate (Fig. 2), and four standard solutions were subjected to stress conditions, which involved storage under destructive conditions like elevated temperature (75 °C), acid environment, basic environment and oxidative condition (H_2O_2 for 24 h). Chromatographic resolution of more than 1.5 was achieved for NAB from unknown peaks.

Figure 2. Chromatograms obtained from (A) a non-spiked stainless steel and (B) the excipient mixture.



Linearity

Standard solutions were analyzed at six different concentration levels ranging from 0.1 to 4.56 µg/mL, with six determinations at each level. Linearity was observed when mean response area was plotted against concentration, using the least square and regression method

Table 1. Linear regression data in the analysis of NAB.

Statistical parameter	Values
Concentration range (µg/mL)	0.1–4.56
Regression equation	y=38782x+33512
Coefficient of determination	r^2=0.996
Residual standard deviation	9373.25

LOD and LOQ

There are several terms that have been used to define LOD and LOQ. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test.

Although reagent package inserts may state that an assay has a dynamic range that extends from zero concentration to some upper limit, typically an assay is simply not capable of accurately measuring analyte concentrations down to zero. Sufficient analyte concentration must be present to produce an analytical signal that can reliably be distinguished from "analytical noise," the signal produced in the absence of analyte

However, some common methods for the estimation of detection and quantitation limit are

- Visual definition
- Calculation from the signal-to-noise ratio (DL and QL correspond to 3 or 2 and 10 times the noise level, respectively)
- Calculation from the standard deviation of the blank
- Calculation from the calibration line at low concentrations

$$DL/QL = \frac{F \times SD}{b}$$

Where

F: Factor of 3.3 and 10 for DL and QL, respectively

SD: Standard deviation of the blank, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression

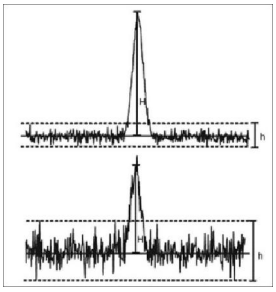
b: Slope of the regression line

The estimated limits should be verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations. The DL or QL and the procedure used for determination, as well as relevant chromatograms, should be reported.

Signal- to-noise

By using the signal-to-noise method, the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. The noise magnitude can be measured either manually on the chromatogram printout or by auto-integrator of the instrument. A signal-to-noise ratio (S/N) of three is generally accepted for estimating LOD and signal-to-noise ratio of ten is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise.

For chromatography a test sample with the analyte at the level at which detection is required or determined is chromatographed over a period of time equivalent to 20 times the peak width at half-height . The signal-to-noise ratio is calculated from the Equation



$$S/D = \frac{2H}{h}$$

where H is the height of the peak, corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, and measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height h is the peak-to-peak background noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained.

This approach is specified in the European Pharmacopoeia. It is important that the system is free from significant baseline drift and/or shifts during this determination.

Shows examples of S/N ratios of 10:1 and 3:1 which approximate the requirements for the QL and DL, respectively. This approach works only for peak height measurements.

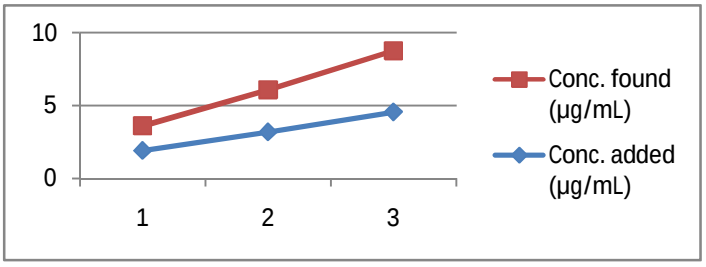
The LOD and LOQ were determined on the basis of standard deviation of the response (*y*-intercept) and the slope of the calibration curve at low concentration levels according to ICH guidelines . The LOD and LOQ for NAB were found to be 0.05 and 0.16 µg/mL, respectively.

Precision and accuracy

Recovery is the percentage of residual material that is actually removed by the sampling technique. Concentration of the analyte was compared with that of the spiked sample at three different concentration levels, 6 replicates each (1.91, 3.18 and 4.56 µg/mL). Observations are reported as relative standard deviation (RSD) and the recovery (%). Observations demonstrate appropriateness of the method for the purpose of residue monitoring.

Table 2. Precision and accuracy of the results obtained from swabbed plates spiked with NAB.

Conc. added (µg/mL)	Conc. found (µg/mL)	95% confidence interval (%)	%Recovery (RSD, *n*=6)
1.91	1.69	88.26–91.54	90.88±0.81
3.18	2.89	90.47–92.84	91.42±1.40
4.56	4.21	90.54–92.89	92.21±0.63



Six consecutive injections of standard solutions on two different days by different analysts and different reagents were performed to evaluate the inter-mediate precision of the method and expressed as the RSD. The RSD was found to be 2.24% and 3.88% for the first and second days, respectively. The observations indicate acceptable inter-mediate precision for NAB solution.

Robustness

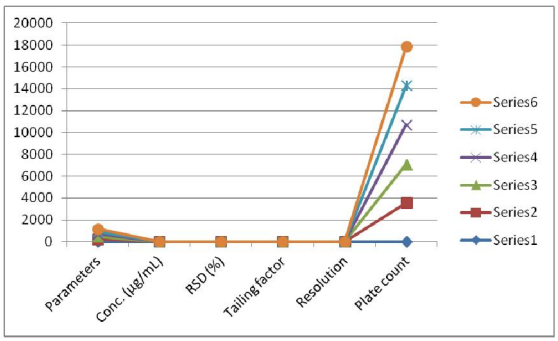
Robustness of the HPLC-DAD method was demonstrated by evaluation of the effect of different chromatographic parameters on the resolution and the concentration of NAB sample. The flow rate was varied from 0.5 mL/min to 1.5 mL/min. The concentration of methanol in the mobile was varied from 52% to 58% and response was recorded at 230±4 nm. Significant differences were not observed in chromatographic parameters.

Table 3. Effect of different chromatographic parameters over method performance.

No.	Parameters	Conc. (µg/mL)	RSD (%)	Tailing factor	Resolution	Plate count
1.	Wavelength (nm)					
	230	0.832	0.69	1.18	2.56	3532
	232	0.835	0.14	1.20	2.44	3545
	234	0.832	0.11	1.20	2.56	3624
	236	0.832	0.83	1.18	2.45	3580
	238	0.834	1.05	1.20	2.56	3573
2.	Mobile phase composition					
	52:33:15	0.831	1.12	1.18	2.11	3360
	53:32:15	0.834	0.34	1.23	2.46	3450
	54:30:16	0.832	0.63	1.22	2.52	3521

No.	Parameters	Conc. (µg/mL)	RSD (%)	Tailing factor	Resolution	Plate count
	56:30:14	0.832	0.92	1.18	2.52	3312
	57:28:15	0.836	0.45	1.22	2.48	3543
3.	Flow rate (mL/min)					
	0.8	0.832	0.54	1.20	2.50	3455
	0.9	0.837	0.66	1.20	2.56	3461
	1.0	0.834	0.32	1.20	2.50	3578
	1.1	0.823	0.92	1.18	2.32	3343
	1.2	0.827	1.32	1.18	2.21	3211

Mobile phase composition shown as methanol:acetonitrile:water, v/v/v.



Sample and standard stability

The stabilities of NAB in the swab matrix and NAB standard solution were tested by storing them at ambient temperature for 24 h. They were injected after 6 h, 12 h and 24 h against fresh standard solutions. The stabilities of the standard NAB solution (4.8 µg/mL) and sample solutions after 24 h showed 2.16% difference in results. The stability of NAB in swab matrix showed 2.87% difference in results. Chromatography of both the samples showed no additional peaks

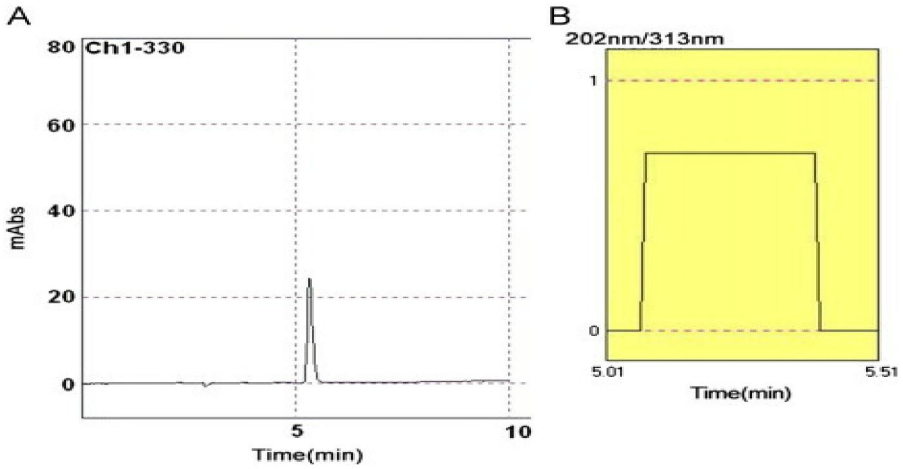


Figure 3. Chromatograms obtained from (A) Nabumetone standard solution, 2 µg/mL, and (B) ratio chromatogram of Nabumetone standard solution.

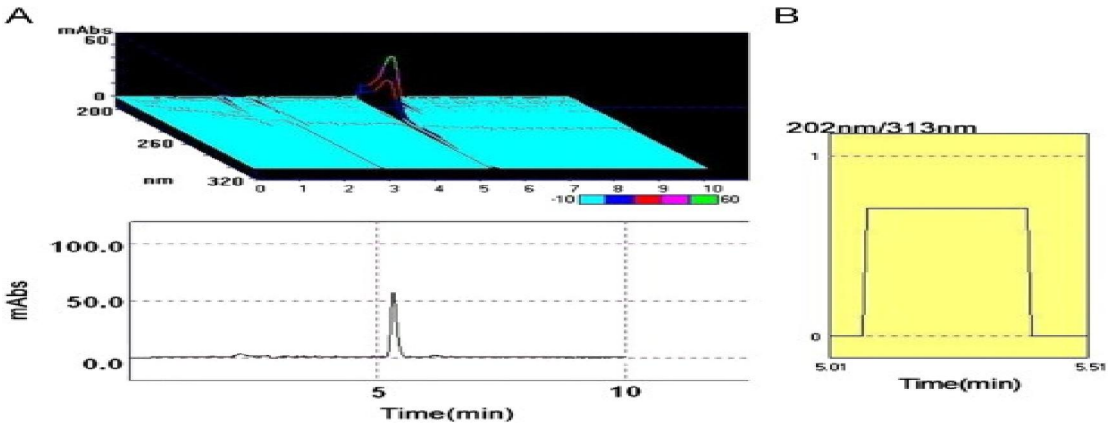


Figure 4. Chromatograms obtained from (A) Nabumetone sample solution, 5 µg/mL, with 3-dimension chromatogram and (B) ratio chromatogram of Nabumetone sample solution.

Filter evaluation

Samples and standard solutions of NAB were filtered with Millipore millex — HV-PVDF 0.45 µm and millex — PTFE-0.45 µm, and compared with unfiltered samples. The Millipore millex — HV-PVDF 0.45 µm and millex — PTFE-0.45 µm pore size syringe filters were qualified for use with filter evaluation ratio 100.28% and 100.36% for NAB standard solution with PVDF and PTFE filters, respectively. For samples, the filter evaluation ratio was 100.28% and 101.13% for PVDF and PTFE filters, respectively.

Estimation of NAB in swab samples collected from production lane

Various samples were collected from different sampling points over the production lane. Samples were tested for residual content of NAB. Partial data are shown in Table

Table 4. Estimation of NAB in actual swab samples (100 cm^2 swabbed area) from different sampling points on production lane.

No.	Sampling point	Residual conc. (µg/mL)
1.	Upper hopper	BDL
2.	Lower hopper	BDL
3.	Die	0.332
4.	Punch	0.362
5.	Lid gasket	0.324

QUALITY CONTROL SUGGESTIONS

For tapelift:

1. Use clear tape, not frosted.
2. Do not fold tape onto itself.
3. Stick tape on the inside of the plastic bag only.
4. Please do not send tape lift samples on slides or cover slips . They may arrive broken making the sample difficult to analyze.

For bulks:

1. Send a representative sample of the specimen if large. This prevents over-handling of the specimen and possible contamination. If analysis of a specific portion of sample is required, please note area(s) or take a tape lift of area.

For swab:

1. For semi-quantitative sampling, the area swabbed needs to be entered on the chain of custody.

For all matrices:

Apply tape, apply swab, or take a small piece of material only from areas where visible mold is seen.

Conclusion

A validated, can be done in selective and simple HPLC-DAD method which was developed for residual determination of NAB to demonstrate the cleaning validation on stainless steel surfaces which related to the production lane. The method which contains the appropriate swab wipe procedure was found to be precise, accurate and linear. There is no any interference from swab solution was observed and samples were stable for 24 hr.

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