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ORIGINAL ARTICLE

Cleaning validation for residual estimation of olmesartan medoxomil on stainless steel surface of pharmaceutical manufacturing equipments using swab sampling and HPLC-DAD method

Nitin Dubey [a,*], Nidhi Dubey [b], Mayank Mandhanya [a], Dinesh Kumar Jain [c]

[a] *Department of Industrial Quality Assurance and Sophisticated Instrumentation, College of Pharmacy, IPS Academy, Indore 452012 (MP), India*
[b] *Department of Pharmaceutical Analysis, School of Pharmacy, Devi Ahilya University, Indore 452011 (MP), India*
[c] *Department of Pharmaceutics, College of Pharmacy, IPS Academy, Indore 452012 (MP), India*

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Abstract Prevention of cross contamination with active pharmaceutical ingredient is crucial and requires special attention in pharmaceutical industry. Current method validation describes residual determination of olmesartan medoxomil (OLME) on stainless steel surface using swab sampling with a sensitive HPLC-DAD analysis. The acceptance limit was decided as 2 µg swab pro 100 cm^2. Cotton swabs impregnated with extraction solution were used to determine residual drug content. Recoveries were 95.81%, 93.06%, and 96%. 24% with RSD below 1.5% at three concentration levels. Residual concentration was found to be linear in the range of 0.557–5.62 µg/mL, when estimated using Phenomenex Luna C_{18} (25 cm × 5 µm × 4.6 mm i.d.) column at 1.0 mL/min flow rate at 258 nm. The mobile phase consisted of a mixture of acetonitrile: methanol: phosphate buffer pH 3.5: tetrahydrofuran (28:13:58:1 v/v/v/v). The LOD and LOQ for OLME were found to be 0.07 and 0.22 µg/mL, respectively. The validated method was found to be simple, selective and sensitive for demonstration of cleaning validation of OLME residues on the stainless steel surface.

* Corresponding author. Tel.: +91 0731 2388922, mobile: +91 9826376242; fax: +91 0731 4041627.
E-mail address: nitindubeympharm@yahoo.com (N. Dubey).
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1. Introduction

Cleaning validation is a critical analytical responsibility of quality assurance system in pharmaceutical industry. Cleaning validation must be demonstrated as per current GMP norms. Residues have a significant cross contamination potential. Residual estimation requires development of selective and sensitive methods capable of quantitative estimation of traces

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remaining over the surface of manufacturing equipments after cleaning procedure. It involves identification of numerous sampling points in the manufacturing lane to demonstrate a complete removal of residues. Currently acceptance limits for residues are not advised by regulatory agencies, but decision is based on the 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 and kept at general limit of 10 ppm.[1–4] 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 residual acceptance criterion. Other approach involves estimating a total amount of allowable residue present on production line, which is termed as residual acceptance level (RAL). Further, 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).

Olmesartan, the medoxomil salt of (5-Methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl}-1H-imidazole-5-carboxylic acid, is the ester prodrug of a new generation of effective and orally active angiotensin-II receptor antagonist (Fig. 1). It blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin-II, one of the most important regulators of blood pressure.[5,6] The determination of OLME from tablet formulation has been carried out by HPLC, HPTLC and spectrophotometrically, 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.[7–9] Available literature revealed that no method related to residual determination of OLME was reported so far, hence it was found worthwhile to determine LSA of OLME 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.

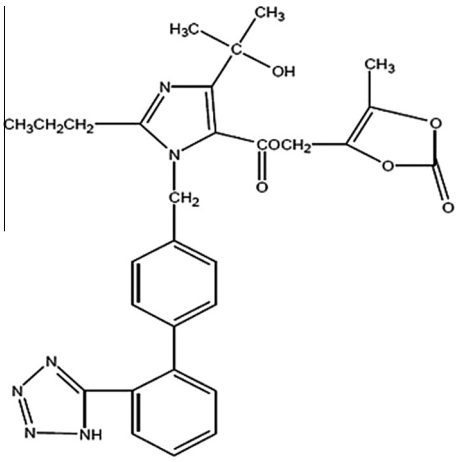

Figure 1 Structural formula of olmesartan medoxomil.

2. Materials and Methods

2.1. Reagent and chemicals

OLME reference standard (USP) was obtained from Cipla Ltd., Mumbai as gratis sample. Olmecip (OLME, 40 mg) and Olmesafe (OLME, 20 mg) tablets were procured from 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. Chemicals such as acetonitrile, methanol, isopropyl alcohol, sodium phosphate, sodium citrate, dibasic potassium phosphate and citric acid were obtained from Merck, Germany. All other chemicals used were of analytical reagent grade.

2.2. Chromatographic system and conditions

The LC system consisted of (Shimadzu LC 10AT VP) gradient pump with universal loop injector (Rheodyne 7725i) of 20 μL injection capacity, 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 software CLASS-Vp (software M-10, version 1.6) (Shimadzu, Tokyo, Japan). Column temperature was ambient. The mobile phase consisted of a mixture of 28 mL acetonitrile, 13 mL methanol, 58 mL phosphate buffer (pH 3.5 ± 0.2, prepared by mixing 0.2 M sodium phosphate solution and 0.1 M citric acid solution at ratio of 30:70 v/v) and 1 mL tetrahydrofuran (28:13:58:1 v/v/v/v). The mobile phase solution was filtered through 0.45 μm membrane filter (Millipore) and degassed prior to use. Extraction solution consisted of 50 mL mobile phase solution, 20 mL IPA and 30 mL water (50:20:30 v/v/v). All chromatographic experiments were performed in isocratic mode. UV detection was performed at 258 nm. The method was validated as per ICH guidelines. The statistical analysis was performed using Microsoft Excel 2007.

2.3. Standard solution preparation

The stock solution of standard was prepared by accurately weighing OLME reference standard and transferred into a 50 mL volumetric flask. Twenty milliliter 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 OLME solutions into separate 100 mL volumetric flasks to get dilutions.

2.4. Sample solution preparation

10 cm × 10 cm of 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: IPA: water, 50:20:30 v/v/v). The swab was squeezed into the swab tube as per the procedure mentioned in Section 2.5. 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.

2.5. Swab wipes sampling protocol

Rinse and swab are two sampling methods available to demonstrate cleaning validation. Swab technique is a preferred technique by the United States Food and Drug Administration. [1,10,11] 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.[12–15] So, a standardized motion protocol is required to establish reproducible recoveries. A patch of 4 × 4 inch swab was immersed in extraction solution and folded diagonally. Excess solution was squeezed to avoid unnecessary dilution of drug. Folded swab was kept between the thumb and second finger, so that necessary force may be applied over the surface through the first finger. The surface was wiped horizontally, starting from outside toward the center. 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.

3. Results and discussion

3.1. 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 pro 100 cm^2.

3.2. Optimization of the 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 28 mL acetonitrile, 13 mL methanol, 58 mL phosphate buffer (pH 3.5 ± 0.2 adjusted with o-phosphoric acid) and 1 mL tetrahydrofuran (28:13:58:1% v/v/v/v). Two-hundred and fifty eight nanometers was selected as detection wavelength as calibration curve showed good linearity and trace level estimations were possible. The above optimized chromatographic conditions are results of efforts to achieve appropriate plate numbers, peak symmetry, resolution and tailing factor.

3.3. Optimization of the sample treatment

Cotton swabs were spiked with different quantities of the drug and placed into tubes. The optimum conditions were achieved with the mobile phase: Isopropyl alcohol: water (50:20:30% v/v/v) as the extracting solvent and sonification time for 15 min.

3.4. Validation of the method

The main objective 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

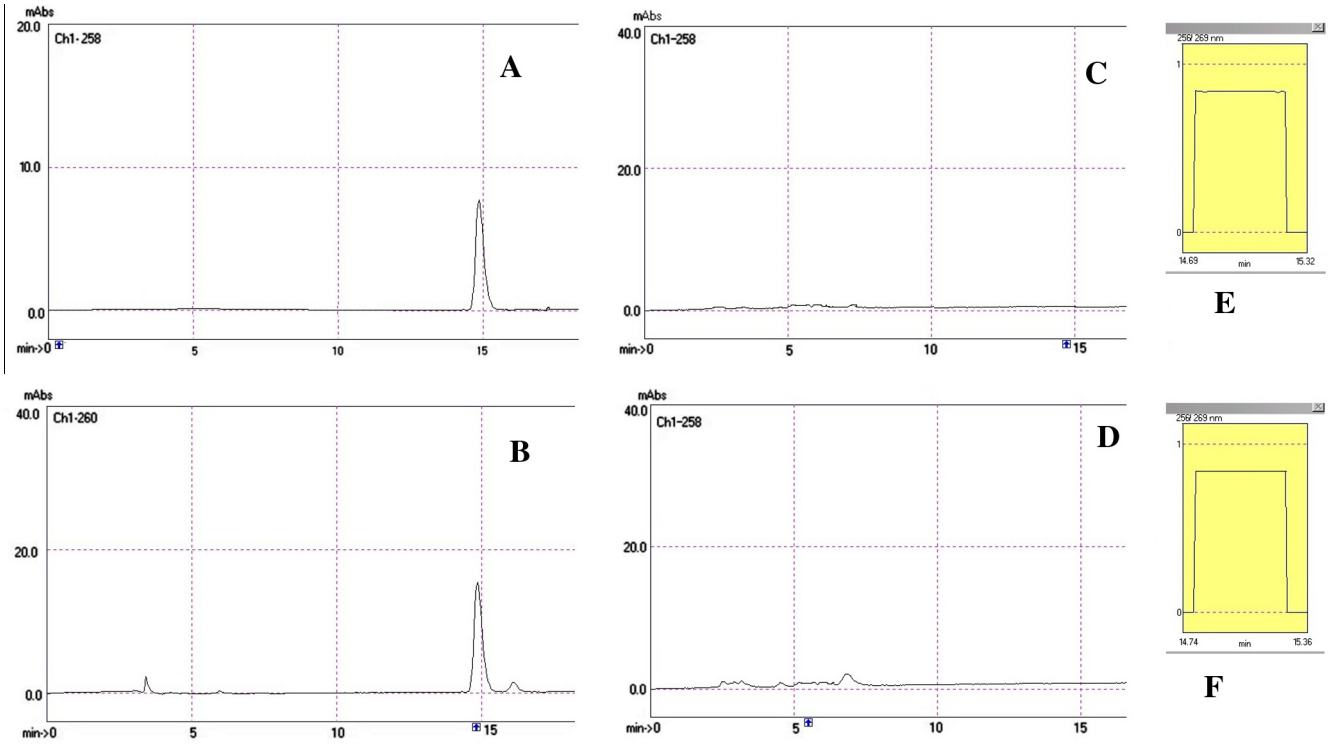

Figure 2 Chromatograms obtained from (a) olmesartan medoxomil standard solution, 20 ppm, (b) olmesartan medoxomil sample, 40 ppm, (c) non-spiked stainless steel, (d) excipient mixture, (e) ratio chromatogram of olmesartan medoxomil sample solution and (f) ratio chromatogram of olmesartan medoxomil standard solution.

Table 1 Linear regression data in the analysis of OLME.

Statistical parameters	Values
Concentration range (μg/mL)	0.557–5.623
Regression equation	$y = 116.99x + 14.619$
Coefficient of determination	$r^2 = 0.9997$
Residual standard deviation	3.85

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

Cons. added (μg/mL)	Cons. found (μg/mL)	95% confidence interval (%)	%Recovery (RSD, $n = 6$)
1.91	1.83	94.2–95.81	95.81 ± 0.81
4.18	3.89	93.47–94.66	93.06 ± 1.4
5.86	5.64	97.54–95.37	96.24 ± 0.63

media. The method was validated for linearity, precision, limit of detection, limit of quantification, accuracy, selectivity, and stability of analyte.[15–17]

3.4.1. 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%.

3.4.2. Specificity

The specificity of the method was checked by using standard, sample, background control sample, negative swab control, swabbed un-spiked stainless steel plate (Fig. 2) and four standard solutions after storage under destructive condition at 75 °C, acid, base and H_2O_2 for 24 h. Chromatographic resolution of more than 1.5 was achieved for OLME from unknown peaks (Fig. 2).

3.4.3. Linearity

Standard solutions were analyzed at six different concentration levels ranging from 0.557 to 5.62 μg/mL, with six determinations at each level. Linearity was observed when mean response area was plotted against concentration using the least square regression method (Table 1).

3.4.4. Limit of detection (LOD) and limit of quantification (LOQ)

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.[18–20] The LOD and LOQ for OLME were found to be 0.07 and 0.22 μg/mL, respectively.

3.4.5. Precision and accuracy

Recovery is the percentage of residual material that is actually removed by the sampling technique. Concentration of analyte was compared with spiked sample at three different concentration levels, six replicates each (1.91, 4.18 and 5.86 μg/mL). Observations are reported (Table 2) as relative standard deviation (RSD) and the recovery (%). Observations demonstrate appropriateness of method for the purpose of residue monitoring.

Six consecutive injections of standard solutions on two different days by different analysts and different reagents were performed to evaluate the intermediate precision of the method and expressed as the RSD. The %RSD was found to be 1.85% and 1.92% for the first and second days, respectively. The observations indicate acceptable inter-mediate precision for OLME solution.

3.4.6. 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 OLME samples (Table 3). The flow rate was varied from 0.5 mL/min to 1.5 mL/min. The concentration of buffer in the mobile was

Table 3 Effect of different chromatographic parameters over method performance.

No.	Parameters	Cons. (μg/mL)	RSD[a]	Tailing factor	Resolution	Plate count
1.	*Wavelength (nm)*					
	254	0.832	0.69	1.18	2.56	3532
	256	0.835	0.14	1.20	2.44	3545
	258	0.832	0.11	1.20	2.56	3624
	260	0.832	0.83	1.18	2.45	3580
	262	0.834	1.05	1.20	2.56	3573
2.	*Mobile phase composition*[b]					
	27:13:56:2	0.831	1.12	1.18	2.11	3360
	27:13:59:1	0.834	0.34	1.23	2.46	3450
	30:13:56:1	0.832	0.63	1.22	2.52	3521
	29:11:59:1	0.832	0.92	1.18	2.52	3312
	26:13:60:1	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

[a] RSD (%).
[b] Mobile phase composition shown as acetonitrile, methanol, phosphate buffer pH 3.5, tetrahydrofuran (v/v/v/v).

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

No.	Sampling point	Residual cons. (μg/mL)
1.	Upper hopper	BDL
2.	Lower hopper	BDL
3.	Lower connecting sleeve	0.24
4.	Tablet collector	0.668
5.	Die	0.332
6.	Punch	0.362
7.	Trunion	0.235
8.	Lid gasket	0.324

varied from 55% to 62% and response was recorded at 258 ± 4 nm. Significant differences were not observed in chromatographic parameters.

3.4.7. Sample and standard stability

The stability of the OLME in the swab matrix and OLME standard solution, was 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 stability of the standard OLME solution (6.2 μg/mL) and samples solutions after 24 h showed 2.73% difference in results. The stability of OLME in swab matrix showed 2.01% difference in results. Chromatography of both the samples showed no additional peaks (Fig. 2).

3.4.8. Filter evaluation

Samples and standard solutions of OLME, 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 a filter evaluation ratio of 100.23% and 100.32% for OLME standard solution with PVDF and PTFE filter, respectively. For samples the filter evaluation ratio was 100.18% and 101.14% for PVDF and PTFE filter respectively.

3.5. Estimation of OLME 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 OLME. Partial data are shown in Table 4.

4. Conclusion

A validated, selective and simple HPLC-DAD method was developed for residual determination of OLME to demonstrate cleaning validation on stainless steel surfaces of the production lane. Method with appropriate swab wipe procedure was found to be precise, accurate and linear. No interference from swab solution was observed and samples were stable for 24 h.

5. Conflict of interest

None.

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