Development and Validation of a Liquid Chromatographic Method for determination of Reserpine Residues for Cleaning Validation in Solid Production Line

PC - 01

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Introduction

The basis of each cleaning validation is a defined cleaning procedure that is described in a cleaning instruction. Critical factors that are taken into consideration during the cleaning validation are the solubility of the drug substance, its tendency towards encrustation and the design of the equipment [1].

The aim of this study was to demonstrate the applicability of HPLC to these purposes by developing, validating and applying an HPLC/UV method to determine the residues of reserpine in support of cleaning control and validation for this antihypertensive drug formulation on the solid production equipment line. Reserpine (RES), chemically known as Methyl 11,17a-dimethoxy-18b-[(3,4,5-trimethoxybenzoyl)oxy]-3b,20a-yohimban-16b-carboxylate, is a Rawolfia alkaloid used in the treatment of hypertension. RES is an official drug in the British Pharmacopoeia (BP).

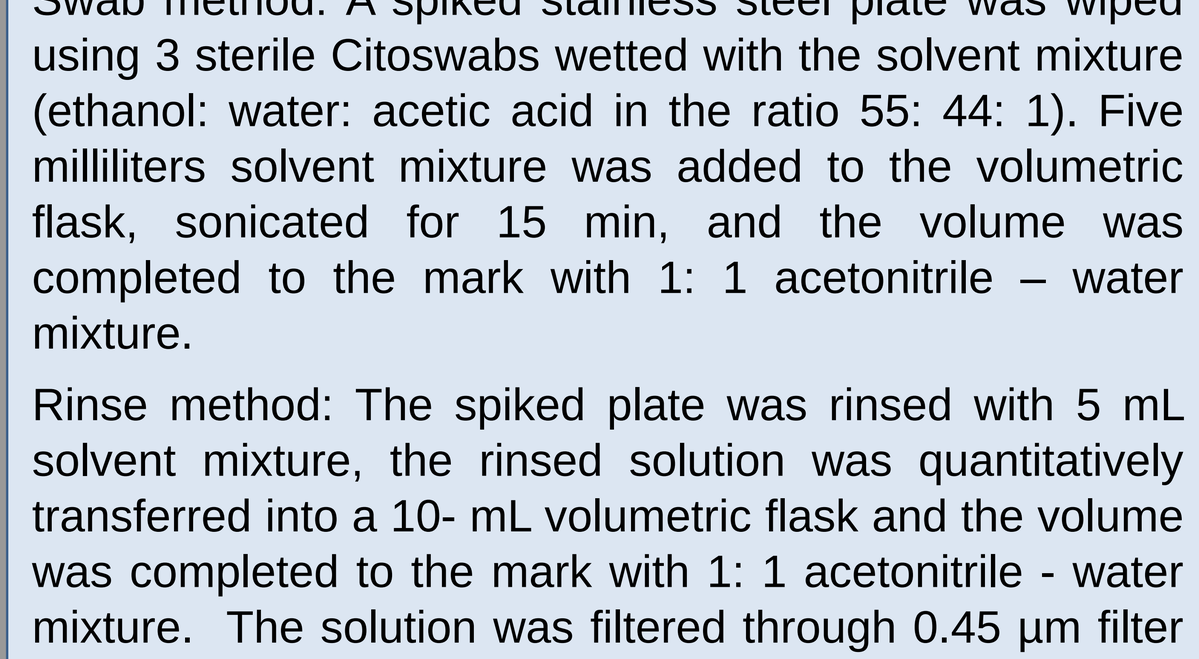
Materials and Methods

All experiments were performed in the isocratic mode. The mobile phase consisted of 1: 1 mixture of acetonitrile and aqueous ammonium chloride solution (1 : 100 w/v), then adjusting the pH of the mobile phase to 5.6 with ammonia. The mobile phase was sonicated and degassed. The flow-rate was set to 1.5 mL/min and the run time is 18 min. The injection volume was 200 µL and the detection wavelength was set at 218 nm.

Preparation of sample solutions: Several stainless steel 316 plates (10 cm × 10 cm) were spiked with 100 µL RES stock solution (8 µg/mL) and were allowed to dry at room temperature for about 10 min to yield a concentration of 0.8 µg RES / plate.

Swab method: A spiked stainless steel plate was wiped using 3 sterile Citoswabs wetted with the solvent mixture (ethanol: water: acetic acid in the ratio 55: 44: 1). Five milliliters solvent mixture was added to the volumetric flask, sonicated for 15 min, and the volume was completed to the mark with 1: 1 acetonitrile – water mixture.

Rinse method: The spiked plate was rinsed with 5 mL solvent mixture, the rinsed solution was quantitatively transferred into a 10- mL volumetric flask and the volume was completed to the mark with 1: 1 acetonitrile - water mixture. The solution was filtered through 0.45 µm filter and was injected into the chromatograph. All solutions are protected from light. (for both swab and rinse method).

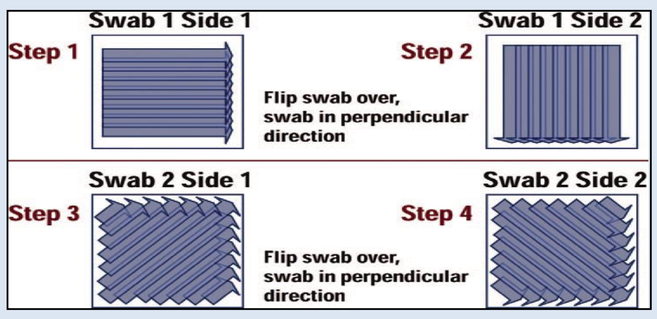


Chromatogram obtained for RES 0.08 µg/mL Rinse solution.

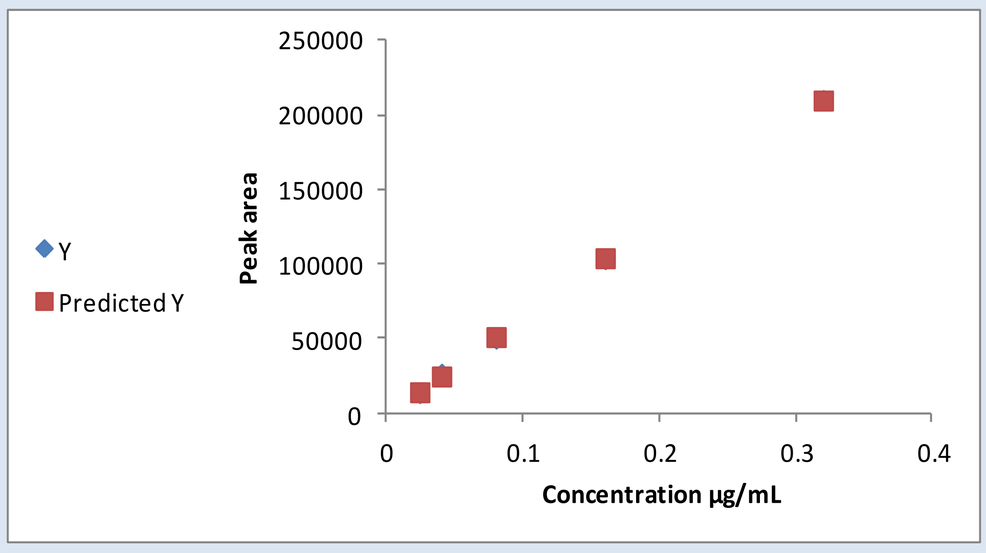
Results

The maximum allowable residue (MAR) was calculated based on both dose criterion and 10 ppm criterion. According to the 10 ppm criterion, not more than 10 ppm of the previously manufactured product is allowed to appear in the subsequent product [1]. In our study the dose criterion was chosen as it gives a much lower MAR. The dose criterion states that no more than (1/1000) of the standard therapeutic daily dose (STD = 0.1 mg) of the contaminant (RES) should appear in the STD of next product calculation procedure.

LA is the acceptance limit, A is the sampling area (100 cm^2) and TA is the total production line area (257249.65 cm^2). LA was calculated to be 0.8 µg.



Calibration of the regression data of peak area and the corresponding concentrations of RES



Recovery of RES from spiked stainless steel 316 surfaces (100 cm^2) applying swab and rinse procedures

RES added (µg/mL)	n = 3			
	Swab method		Rinse method	
	% Recovery	Systematic error	% Recovery	Systematic error
0.04	76.99	-23.01	85.80	-14.20
0.08	79.96	-20.04	81.12	-18.88
0.32	78.03	-21.97	83.65	-16.35

Conclusions

A rapid and sensitive reversed-phase high performance liquid chromatographic method to determine trace levels of RES in rinse and swab samples collected from the equipment surfaces has been developed and found to be accurate and precise. The method was validated for specificity, limit of detection, recovery, precision, and stability of standard and sample solutions. In addition, the stability of the swab samples with analyte was evaluated to determine the allowable time interval between sampling equipment surfaces and extraction of the analyte with sample solvent. The swab material, solvent from wetted swab, rinsing solvent as well as excipients of the commercial sample did not interfere in the analysis, which proved the specificity of the method.

References

GMP Guidelines Good manufacturing practice & implementation knowledge for assay implementation of Good manufacturing practice Important GMP regulation and guidelines of EU, PIC/S and ICH.

The British Pharmacopoeia, Her Majesty’s Stationery Office: London, 2012, electronic version.