# Densitometric Determination of Ondansetron Impurity B in Pharmaceutical Formulation

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BACKGROUND

Ondansetron hydrochloride is a widely used drug in nauseas and vomit prevention during chemotherapy and radiotherapy. Impurity B is the potential drug substance impurity. This impurity is also included in USP and Ph.Eur. monographs for Ondansetron Hydrochloride and Ondansetron Hydrochloride Dihydrate substance.
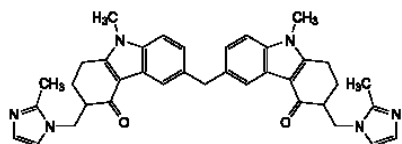
Impurity B = 6,6′-methylenebis[(3RS)-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4H-carbazol-4-one]

PHARMACOPOEIAL METHODS

For determination of impurity B (limit 0.4%) USP and Eur.Ph pharmacopoeia prescribe TLC method with visual detection under UV light. In USP method mobile phase is consisting of chlorophorm, ethyl acetate, methanol and ammonium hydroxide (90:50:40:1, v/v) and sample is dissolved in methanol. In Ph Eur method mobile phase is consisting of methylene chloride, ethylacetate, methanol and concentrated ammonia (90:50:40:2 v/v) and sample is dissolved in mobile phase. Stationary phase is TLC silica gel plate F254, 10x10 cm and evaluation is under short-wavelength UV light (254nm). Unfortunately both methods have proven to be unsuitable for determination of impurity B in Ondansetron injection because of the placebo influence.
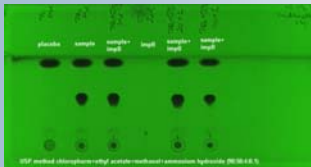

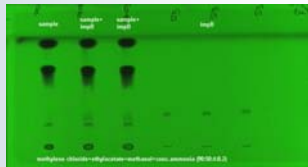Figure 1. Chromatogram of Ondansetron injections, 2mg/ml, according to USP

Figure 2. Chromatogram of Ondansetron injections, 2mg/ml, according to Eur.Ph.

DEVELOPMENT OF NEW METHOD

Separations of placebo, ondansetron and impurity peaks were accomplished by modification of the mobile phase composition and selection of the right plate. By varying the concentration of chlorophorm and ammonia in the mobile phase the best resolution was obtained with ratio 18:5:4:0.15, v/v. Concentration of the ondansetron in sample solution (2 mg/ml) was much lower then in pharmacopoeial methods (12.5 mg/ml) so TLC silica gel F254 have been replaced with HPTLC plates that can give us lower detection limit, better sensitivity and better resolution. To avoid problems with aquatic sample solution HPTLC silica gel plate 60 WR F254s special resistant to water was choosen instead of normal HPTLC silica gel 60 F254 plate. To enable detection of 0.1% of the impurity B concentration, detecting mode was changed from visible detecting to densitometrically detection on 311 nm which is determined as the maximum of the spectra of impurity B and ondansetron. Saturation of the chamber with plate 30 min prior to development was applied to get a better repeatability.
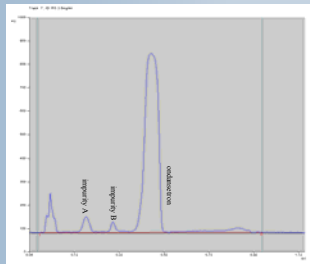

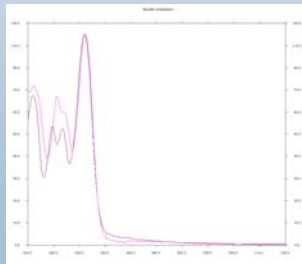Figure 3. Chromatogram obtained with sample solution at 311

Figure 4. Spectra of Ondansetron hydrochloride and impurity B with maximum at 311 nm
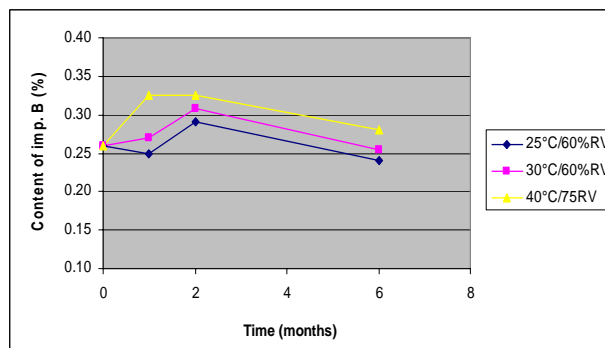
VALIDATION

Performance of the method is validated through selectivity, linearity, accuracy, precision and limit of detection and quantification, robustness.

Validation parameters	HPTLC method
Linearity Range Correlation coefficient	0.05 – 1.0% 0.998
Accuracy Range Recovery	0.1 – 0.6 % 94.5 %
Precision a) measurement repeatability b) sample application repeatibility c) intermediate precision	RSD = 1.1 % RSD = 8.1 % RSD = 12.3 %
Stability a) in solution b) on the plate	at least 24 h at least 2 h

Validation parameters	HPTLC method
Detection limit Quantification limit	0.03% 0.1%
Selectivity HCl, NaOH, H₂O₂, light, temp.	No interferences between peaks
Robustness	Variation of saturation time, temp. and mobile phase composit. do not influence the quality of chromatographic separation.

RESULTS

Stability of ondansetron pharmaceutical formulation was performed and content of impurity B was analyzed using quantitative HPTLC method.

CONCLUSION

- Performance of the method is validated through selectivity, stability, linearity, accuracy, precision and limit of detection and quantification. No interactions of placebo or degradation products of Ondansetrone hydrochloride are observed during stability testing (stress test).
- We can conclude that It can be used as stability indicating method for determination of impurity B not only in the substances but also and in pharmaceutical formulations.