

Development of HPLC determination of related substances in a new CNC agent – 1-(4-methoxyphenyl)-5-[2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxo-ethyl]pyrazolo[3,4-d]pyrimidin-4-one

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Introduction. In previous studies a novel CNC agent, 1-(4-methoxyphenyl)-5-[2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxo-ethyl]pyrazolo[3,4-d]pyrimidin-4-one was synthesized and studied. An integral part of a new API pharmaceutical development is identification of impurities that may adversely affect pharmaco-technological parameters, pharmaco-toxicological profile, and cause the API or dosage forms side effects. International Conference on Harmonization guidelines Q3A contains recommendations concerning the content, identification and qualification of impurities in new APIs obtained by chemical synthesis [1,2]. The purpose of the presented study was development and validation of the method for determination of related impurities of new CNS agent with a broad spectrum of neurotropic activity using HPLC method.

Materials and methods. Liquid chromatography separation was performed using a Shimadzu Nexera X2 LC-30AD HPLC system (Shimadzu, Japan) composed of a quaternary pump, an on-line degasser, a column temperature controller, the SIL-30AC autosampler (Shimadzu, Japan); the CTO-20AC thermostat (Shimadzu, Japan) as well as the SPD-M20A diode array detector (DAD).

Results and discussion. Potential impurities at all stages of synthesis were determined and synthesised: ethyl 5-amino-1-(4-methoxyphenyl)-pyrazole-4-carboxylate (A) – the first main synthesis intermediate, 1-(4-methoxyphenyl)-1,5-dihydro-pyrazolo[3,4-d]pyrimidine-4-one (B) – the second one, chloro-1-[4-(4-methoxyphenyl)piperazin-1-yl]ethanone (C) – alkylating agent adding at the last stage of synthesis. Freshly prepared solutions were put into the HPLC system with DAD detector, and UV-spectra were recorded. The maximum UV-absorption of the studied compounds was detected at the same wavelength – about 240 nm: 1-(4-methoxyphenyl)-5-[2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxo-ethyl]pyrazolo[3,4-d]pyrimidin-4-one – 236 nm, impurity A – 238 nm, impurity B – 237 nm, impurity C – 242 nm. Chromatographic conditions: chromatographic separation was performed on an ACE C18 column 250 mm x 4.6 mm, 5 µm particle size with a pre-column. The mobile phase A – 2.0 g/L sodium phosphate dibasic solution with 5.0 ml triethylamine, adjusted to pH 7.0 with dilute phosphoric acid R; the mobile phase B – methanol R; gradient program 0-15 min 65→25% A, 35→75% B; 15-17 min 25% A, 75% B; 17-18 min 25→65% A, 75→35% B; 18-25 min 65% A, 35% B. The flow rate was maintained at 1 ml/min with UV detection at 240 nm. The sample injection volume was 10 µl and the column temperature was maintained at 45 °C. All solutions were prepared immediately before use according to the procedures described below.

Conclusions. A new HPLC method has been developed and validated for the determination of related substances for new perspective CNC agent. The developed method is specific, accurate, precise and linear across the analytical range according to ICH recommendations.

References

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2. ICH Harmonised Tripartite Guideline. Impurities in new drug substances Q3A (R2). In: *Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use*, Geneva, Switzerland. 2006.