UNIVERZITA KARLOVA V PRAZE FARMACEUTICKÁ FAKULTA V HRADCI KRÁLOVÉ

Téma diplomové práce	**Determination of related compounds of bromhexine hydrochloride active substance using ultra high-performance liquid chromatography**
Jméno studenta, studentky	**Maltina Shkodra**
Jméno oponenta	**PharmDr. Radim Kučera, Ph.D.**

II. Posudek oponenta

Maltina Shkodra dealt in her diploma thesis with the transfer of a pharmacopoeial HPLC method to a UHPLC system. She optimised temperature and flow rate and assessed the influence of these parameters on the chromatographic performance. The author focused on the reduction of time and solvents consumption.

The diploma thesis has 55 pages and 43 references are cited. The theoretical part is devoted to description of chromatographic parameters, equipment, columns, UHPLC facility and validation procedures. The description of experiments and obtained results are summarised in the chapter Experimental. The results are documented by figures and tables.

I have the following comments:

The graphics of the thesis is sub-standard (the text is not formatted, double spacing is used, tables are presented as figures, some figures and formulas are of poor quality, the peaks in chromatograms are not identified, etc.). The author did not cite the sources of some figures; some references are missing in the text, especially the reference to the ICH validation guideline. I also missed the description of transferred method in the text. I think that the whole text deserved more attention in order to avoid some formal and factual mistakes and inaccuracies. I recommend attaching errata.

I have the following questions:

p. 12 – How could be determined the dead volume on C18 stationary phases? Is really the retention factor distinct for every compound? What is the recommended range for retention factor?

p. 13 – How can you manipulate with the composition of the stationary phase?

p.14 – Is it possible to perform the HPLC separation above 60°C?

Eq. 7 – W_1, W_2 - are not widths at half height.

p. 19 – could be operated RP silica-based stationary phases in pH above 8? Why are the stationary phases endcapped?

The pH and temperature stability of polymeric and zirconia phases is generally different than it is stated in this thesis.

p. 21 – Could you please compare the elution strength of methanol, acetonitrile and tetrahydrofuran?

p.24 – Is the term "mass spectroscopy" correct? Can you describe the principle of "API ionization"?

p. 26 – Despite many positive features – can you describe the main disadvantage of UHPLC columns?

p. 30 – Is the expression of relative standard deviation is correct?

pp. 30-32 – How can be performed the repeatability test according to ICH? Is there a difference between reproducibility and intermediate precision? How is usually expressed the linearity? In which range is performed the linearity test for impurities according to ICH?
p. 36 – could you please describe the difference between a common C18 phase and the phase used in this work?
p. 39 – Why did you select the flow rate 0.5 mL/min. and 40°C? Why was injected 1.6 µL? Meet the peak symmetry the pharmacopoeial criteria?
p. 50 – Was the resolution in the transferred method 23?

Based on your results which quantification method would you choose for the evaluation of bromhexine impurities?

Navrhovaná klasifikace **good**

V Hradci Králové dne 31.5.2012

Podpis oponenta diplomové práce