## Abstract

This work extends the graduation thesis which found optimal chromatographic conditions for a separation of paracetamol, caffeine and propyphenazon, and the impurity p-aminophenol in the pharmaceutical preparation Valetol by HPLC method.

At first, the benzoic acid was found as a suitable internal standard for the quantification. Next, the analytical method validation was carried out, including the suitability test of the chromatographic system.

Within the suitability test of the chromatographic system the following attributes were tested:

• column efficiency expressed as a number of theoretic plates N
• assymetry of chromatographic peaks expressed as factor T
• resolution of chromatographic peaks
• reproducibility expressed as a relative standard error

Within the analytic method validation were tested:

• precision
• linearity
• accuracy
• selectivity
• robustness
• stability

The robustness test confirmed the analysis sensitivity to the change in the character of the mobil phase. As a part of the robustness test two parameters of mobile phase were tested: pH and triethylamine concentration changes. It was confirmed that the change of pH of the mobile phase influences mostly the retention times of p-aminophenol and benzoic acid. The change of triethylamine amount in the mobile phase influences mostly the retention time of p-aminophenol and the area under the peak (its quantification).

Demands for p-aminophenol in methanol-solution stability were not fulfilled for any of the tested storage conditions, and its considerable unstability was proved. It is essential that fresh p-aminophenol methanol-solution should be prepared every day.

The analytic method validation shows that it does not fulfil the robustness and stability attributes. Thereafter the method can be used in everyday routine, but a special attention must be paid to the facts shown above.