7. General Conclusion

The CMC has been successfully coupled with HPLC for the determination of ethynilestradiol and levonorgestrel in pharmaceutical preparations. The couple of the CMC with HPLC developed here takes advantage of integrating the three processes of continuous extraction, preconcentration, and separation in the CMC and provides a suitable method for determination of ethynilestradiol and levonorgestrel in pharmaceutical preparations. The proposed method shows a good performance based on the evaluation of several factors (i.e. enrichment factor, linearity, reproducibility, accuracy, and sensitivity) and requires a quite simple instrumentation.

In optimum condition, the enrichment factor of 71.56 (LEV) and of 72 (ETE) were achieved by introducing 10 mL of sample with the end volume of 100 µL. Good linearity ($r^2>0.99$) was observed. The relative standard deviation (<3%) indicated that the method has very good reproducibility. The accuracy of the method was indicated by recovery percentages. Satisfactory recoveries were observed for LEV (96.24%; n=10) and for ETE (103.13%; n=20) from measurement of a single standard solution of 72.1 ng mL$^{-1}$ and 60 ng mL$^{-1}$. In case of sensitivity, the HPLC coupled with the CMC showed even better sensitivity which is indicated by lower detection limit. By applying the HPLC coupled with the CMC, LEV is 76.92 times more sensitive (from 20 ng mL$^{-1}$ to 0.26 ng mL$^{-1}$) and ETE is 105.1 more sensitive (from 49.4 ng mL$^{-1}$ to 0.47 ng mL$^{-1}$) than the standard method. Much higher sensitivity and enrichment factor are even obtained, when the sample volume is increased.
In general, the results obtained using the HPLC coupled with the CMC on determination of LEV and ETE in pharmaceutical preparations were comparable with those obtained by the HPLC standard method suggested by USP. Applications of the method on the analysis of those compounds in pharmaceutical preparations were found to be in good agreement with the certified content. The recoveries of LEV and ETE from commercial pharmaceuticals vary between 90.27 and 107.67% which meet the requirement for LEV and ETE tablets specified in the USP.

The couple of the CMC and spectrophotometer for the determination of zinc and copper in pharmaceutical preparations is also successful. The proposed method allows the combination of subsequent extraction, preconcentration, separation, and spectrophotometric determination of the samples. Besides, it allows automatically measurements by applying a software package for computer-aided flow analysis, device control and data acquisition. Moreover, It is cheap because it uses microliters of the reagents and solutions per analytical cycle, readily available equipment, and very simple to assemble and operate.

The main advantage of the proposed system is the flexibility of the system with a very cheap cost per sample. Preconcentration of the target compound can be carried out by changing either the flow rate of the sample or the preconcentration time. The analytical throughput of this method can be increased by increasing the flow rate of the sample and reducing the preconcentration time.

The proposed method proves a good performance which is indicated by several parameters: the enrichment factor, linearity, reproducibility,
accuracy, and sensitivity of the method. In the optimum conditions, the enrichment factor of 5 was achieved by introducing 0.825 mL of the sample. Good linearity of zinc ($r^2>0.9961$) and copper ($r^2>0.9968$) were observed. The relative standard deviation (<3%) indicated that the method has very good reproducibility.

The accuracy of the method was indicated by recovery percentages. Satisfactory recoveries were observed for zinc (98.8-100.3%) and copper (99.6-101.2%) from measurement of a reference standard solution which indicated a good accuracy. The proposed method has been successfully applied for the determination of zinc and copper in pharmaceutical preparations. And the results are found to be in good agreement with the certified content with the percent recoveries lie between 95.6 to 99.9%.