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# NOVEL ELUENT COMPONENTS IN HPLC-ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Using mass spectrometric (MS) detection for liquid chromatography (LC) sets limitations for eluent choice.

Pharmaceutical compounds often contain amine groups, which give it alkaline properties. Separation of alkaline compounds by HPLC is often better accomplished using basic buffer solutions. For LC-MS applications selection of buffer components is limited. These compounds must be volatile and should not suppress the ionization of the analyte. [1] Separation of five antibiotics norfluoxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFL), sulfamethoxazole (SMX) and sulfadimethoxine (SDM) with acidic eluent was problematic [2]. In the present study two fluoroalcohols 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol (HFTB) were used as buffer solution components for antibiotics separation by LC-MS. Present work is the first report of using HFIP and HFTB in alkaline (pH 9 and 10) buffer solutions. Influence of pH change for separation of the antibiotics was under close study.

Present study showed better separation and efficient electrospray ionization for NOR, CIP, OFL, SMX and SDM with HFIP and HFTB compared to known LC-MS buffers in high pH range such as ammonium acetate (pH 9 and 10) or 1-methylpiperidine (pH 9.85). All buffer solutions were tested in same LC-MS gradient elution conditions with methanol. Influence of two additives for buffer solutions triethylamine (TEA) and  $\text{NH}_4\text{OH}$  were studied. It appeared that using  $\text{NH}_4\text{OH}$  additive in buffer solution with HFIP increases analytes ionization (Fig. 1).

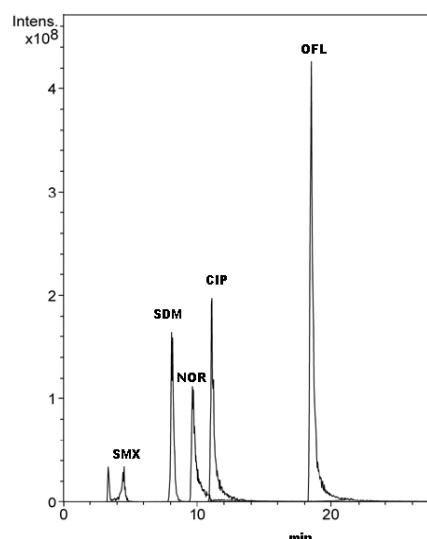


Fig. 1. Separation of five antibiotics. Eluent 5 mM HFIP (pH=9.0 adjusted with  $\text{NH}_4\text{OH}$ ).

Using HFIP with  $\text{NH}_4\text{OH}$  at pH=9 as buffer solution was applied for antibiotics determination in lettuce samples.

## References

1. Snyder, L.R., Kirkland, J. J., Glajch, J. L. Practical HPLC method development, 2<sup>nd</sup> ed.; Wiley, New York, 1997.
2. Lillenber, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R., Nei, L., Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. *J. Chrom. A.* **2009**, 1216, 5949-5954.