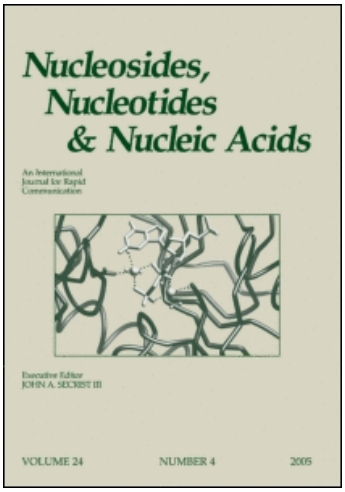

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HPLC and LC-MS of Nucleosides

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HPLC AND LC-MS OF NUCLEOSIDES.

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Summary.
An LC-MS system for the analysis and unambigious identification of nucleosides is described using a microbore column and volatile buffer systems. The detection of pseudouridine in normal urine was used as a test for diagnostic applications.

For many years the area of nucleic acid research was hindered by the lack of good analytical techniques. Since nucleosides and nucleotides are heat-labile and low -volatile, gas chromatography cannot be used without derivatisation[1].

Development of high presssure liquid chromatography(HPLC) has proven to be very valuable for such separations. Originally low pressure ion exchange chromatography was used,but subsequently HPLC has proven to be particularly suitable using isocratic conditions as well as gradient elution[1,2].

However,in order for a chromatographic technique to be useful, peaks in biological samples must be identified unambigiously, for example by combining several methods such as retention times, use of standars, isotopic labelling,enzymic peak shift procedures or by using one single identification technique: mass spectrometry. Soft ionization techniques such as field desorption-,desorption/chemical ionization-,fast atom bombardment- and liquid chromatography-mass spectrometry(LC-MS) have proven to be most useful in the structure elucidation of such compounds. Amongst these mass spectrometric techniques LC-MS occupies a special place since it can be used for direct on-line analysis of mixtures. Experiments described in this area include nucleoside and nucleotide analysis via thermospray,moving belt and direct liquid introduction(DLI) interfacing. The thermospray interface gives good results not only

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in the nucleoside but also in the nucleotide field[3,4]. LC-MS using the moving belt interface seems to reveal molecular weight information if spectra are taken under chemical ionization conditions using ammonia as the reagent gas[5].

DLI-interfacing of a mass spectrometer with a liquid chromatograph imposes specific restrictions upon the operating conditions of both HPLC and mass spectrometric part of the combined LC-MS system. This means that special conditions ,using volatile salts such as ammonium acetate,ammonium formate etc. had to be established[6,7]. Furthermore ,due to cluster ion formation in the ion source,effluents must be chosen as a function of the molecular weight of the molecules to be determined. Since for most nucleosides a mass rande from m/z=110 to m/z=350 has to be covered,the choice of organic solvents in HPLC is restricted towards methanol and acetonitrile. Even then,it is impossible to detect ions below m/z=130,unless a small oven is mounted between the DLI-probe and the ion source. Such a desolvation chamber was built in our workshop after the description given by Dedieu et al.[8] but with slight modifications of the heating system. The impact of the insertion of this chamber into the DLI/LC-MS system was studied in combination with a μ-Bondapack 10RP18 column(30 cmx 3.9mm) using standard mixtures (30 μg) of (deoxy)nucleosides at a flow-rate of 1cc/min. Mass spectra could now be recorded from m/z=110 or m/z=115 depending upon the % of methanol in the effluent. As a result strucural important fragmentions such a $[BH + H]^+$,$[B + 30]^+$,$[B + 44]^+$ and $[B + 44 - 16]^+$(deoxyribonucleosides) could now be observed.

Some kinds of deseases (gout or cancer) may give rise to "higher" levels of nucleosides in i.e. blood or urine. In order to obtain diagnostically interesting analysis of such urines,the detection limit of the system had to be improved. This was done by using a 50cmx1mm (Chrompack) 10 RP18 column operated at a flow-rate of 70 μl/min. This flow-rate implies a split factor of 1/7 instead of 1/100 if flow-rates of 1cc/min were used. Under theseconditions some severe difficulties had to be overcome due to corrosion of the nickel diaphragm in the interface by the effluent. Once these difficulties were solved,good separations and spectra were obtained for standerd mixtures (1.5 μg) of cytidine, uridine,2'-deoxycytidine e.g.,using 97% 0.01M NH_4OOCH/3%CH_3OH as the effluent.

Finally 20 cc of morning urine was cleaned-up[2] using Affigel 601 (Biorad) and analyzed on the microbore DLI/LC-MS system using the same chromatographic conditions as described above. Pseudouridine could be identified by monitoring m/z=245 (MH^+) and by comparison with the mass spectrum of a pseudouridine standard. The possibilities of this technique will now be studied for the analysis of pathalogical urine samples.

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