Requirements to Solvents in LC/MS

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Abstract ..... The influence of different ions on Electrospray LC/MS is shown. Both spectral behaviour and sensitivity can be changed dramatically in the presence of either alkali ions or organic acids. This may affect the analytical performance, or even make it impossible.

The concentration influence of Na^+- and K^+-ions on mass spectra of peptides is shown with human gastrin as a sensitive model peptide. Alkali metal ions form clusters (adducts) with peptide molecules, such as $[M+H+Na]^{2+}$, which become dominating at alkali concentrations of 5 ppm and higher. The sensitivity is decreased 10-fold, and a correct mass assignment for subsequent MS/MS experiments is complicated. Carbohydrates and glycosides have high affinity to Na^+-ions and preferably form $[M+Na]^+$-ions. Addition of sodium salts may therefore help to increase the sensitivity as compared to protonation with organic acids. In most standard applications addition of organic acids like formic or acetic acid increases the formation of $[M+H]^+$-ions, but trifluoric acetic acid (TFA) acts as a strong suppressor, that has to be counteracted with formic acid to achieve enough sensitivity.

1. Ion formation in electrospray..... Electrospray is today the most widely used ionization method in LC/MS. This is valid especially for proteins, peptides and many pharmaceutical active substances, at least when they are polar. Since the first experiments associated with liquid ionization, scientists tried to understand the mechanism and to set up a model, which fits results best. The basic phenomenon, formation and behaviour of charged droplets, was first desribed in 1917 by Zeleny [1], and investigated in more detail in 1955 by Drozin [2]. This study was used in pioneering work for an electrospray interface by Dole et al. [3,4] and continued in 1984 by Yamashita and Fenn [5,6], which resulted in a description of an LC/MS interface in 1985 [7].

Since the early days of electrospray, theories of ion formation have been discussed. The hypothesis of Dole [3], based on the assumption that the observed ions are produced by simply desolvating the charged droplets, is called the „charge residue model". Roellgen et al. [8] postulated a „soft desolvation" of ions by solvent evaporation from small charged droplets by either electrohydrodynamic or mechanical instabilities (or both). These theories led to the present model of a „coulomb explosion", where the charge stays with the analyte when a charged droplet becomes smaller and eventually „explodes", losing all neutral solvent molecules [9]. But when different ions are present, i.e. from the solvent, they can compete for addition to the uncharged analyte. This is especially true in HPLC, when using buffers, or in CE, where salts are mandatory. Wang and Cole [10] investigated the influence of ionic strength on the positive-ion electrospray mass spectra of myoglobin and reported a 10-fold decrease in signal intensity and almost no change of charge-state distribution, when increasing the salt concentration. The aim of our work was to study the influence of different ionic additives on ion formation, sensitivity and spectral behaviour of three different model molecules, each standing for a group of substances.

2. Results: human gastrin ..... Human gastrin is a peptide with 17 amino acids (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2). An aqueous stock solution (100µg/ml) was diluted with water or water/methanol of known Na^+/K^+-content. The chosen concentrations cover a range from 0.01 to 900 mg/kg (ppm). At concentrations below 0.1 ppm the preferred ionization product is $[M+2H]^{2+}$, which appears as the base peak. Sodium and potassium ions form $[M+H+Na]^{2+}$ and $[M+H+K]^{2+}$ adducts in low abundance (Fig. 1). With an alkali ion content near 10 ppm, the relative abundance of the doubly-protonated molecule ion is decreased, the total response (ion yield) for the peptide is decreased 10-fold, and the sodium ion adducts become dominant with $[M+2Na]^{2+}$ as the most abundant ion within the doubly-charged group (Fig. 2). In this spectrum it is almost impossible to assign the correct molecular ion for subsequent MS/MS experiments (amino acid sequence). The used model peptide human gastrin contains 5 acidic Glu-residues and is therefore very susceptible for that effect. It must taken into account that in structure elucidation of unknown proteins, the resulting peptides cannot be foreseen. For that reason samples have to be desalted thoroughly prior to MS, and solvents should have a very low alkali content.

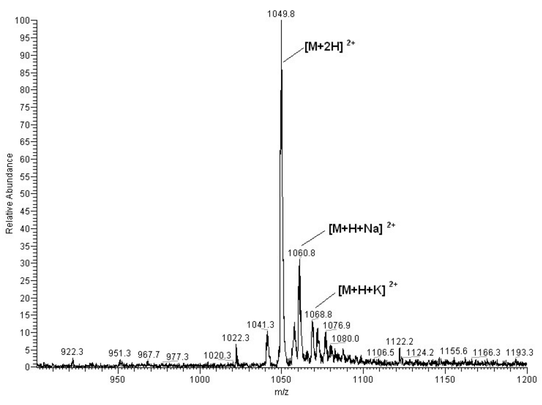

Fig. 1: ESI-MS of human gastrin (M=2097) in water (0.2% formic acid) with 0.10 ppm Na^+ and 0.09 ppm K^+. Only few adducts are observed, the doubly-charged molecule ion can be determined without doubt. Instrument / conditions: human gastrin (10 ng/µl), direct infusion (4µl/min), LCQ Advantage (Thermo Electron, Dreieich, Germany) equipped with electrospray interface.

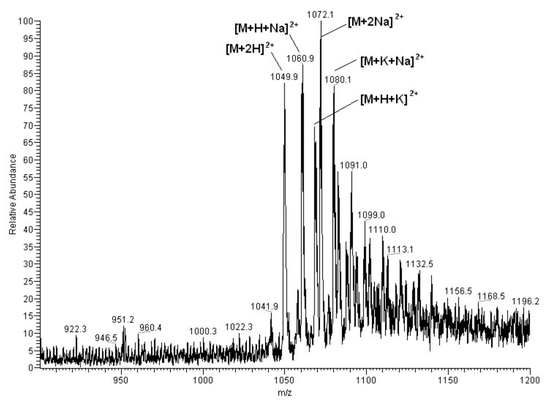

Fig. 2: ESI-MS of human gastrin (M=2097) in water (0.2% formic acid) with 9.0 ppm Na^+ and 8.4 ppm K^+. Clusters with alkali ions are dominating. The most abundant peak is the doubly-charged sodium adduct $[M+2Na]^{2+}$, the absolute sensitivity is decreased 10-fold. Instrument / conditions same as Fig. 1.

3. Results: reserpine ..... Reserpine is an alkaloid and pharmaceutical active compound. This substance of known for its „normal" behaviour and is therefore often used as a standard in LC/MS. The effect of protonation with different organic acids [11] can be demonstrated very good with this molecule. Compared to pure water the addition of either formic (FA) or acetic acid (AA) increase the ion yield (sensitivity) in electrospray mass spectrometry (Fig. 3). A mixture with acetonitrile (50/50, v/v) is even more efficient. The negative (suppressor) influence of trifluoro acetic (TFA) acid, which is already described by Eshraghi and Chowdhury [12], is also evident, with signals below the sensitivity obtained without any additive. In addition, the normal $[M+H]^+$-ion with 609.3 dalton is changed to 607.3 dalton (Fig. 4). When formic acid (0.05% TFA, 0.05% FA) is added, the molecular ion is back to 609.3 dalton, but still at a very bad sensitivity level. Adding only 0.01% TFA to 0.1% FA still results in a sensitivity notedly below the level of pure water. Ammonium acetate does not ionize reserpine in a useful way; unspecific fragments occur at poor sensitivity.

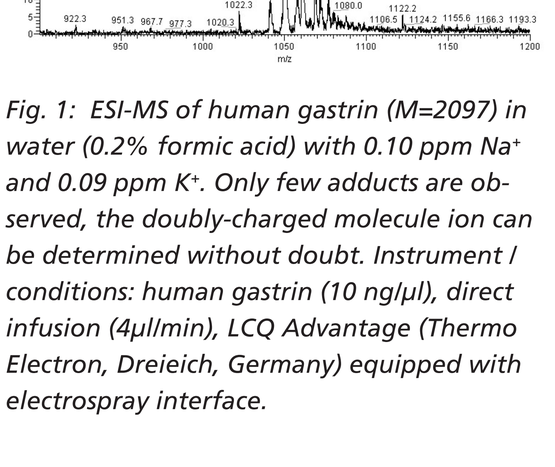

Fig. 3: Ion yield of reserpine with different ionizer additives.

4. Results: digoxin ..... Digoxin is a glycoside, containing 3 digitoxose molecules on a steroid-like aglycone. It is pharmaceutical relevant as an antiarrhythmic drug at a very low blood concentration (5ng/ml). Monitoring with normal HPLC is not possible due to lack in sensitivity. LC/MS is much more sensitive and selective in addition. The ion yield of digoxin is shown in Fig. 5, being most efficient with the addition of sodium citrate. Even with the addition of formic acid (FA) or ammonium acetate the resulting molecular ion is $[M+Na]^+$, which can expectedly be boosted by addition of a sodium salt. Although fragmentation is also higher, sensitivity can be increased remarkably. The fragmentation pattern (Fig. 6) shows very clearly a successive breakdown of digitoxose units (M=130 dalton). These results are in good agreement with a recent work of Sudergat et al.[13].

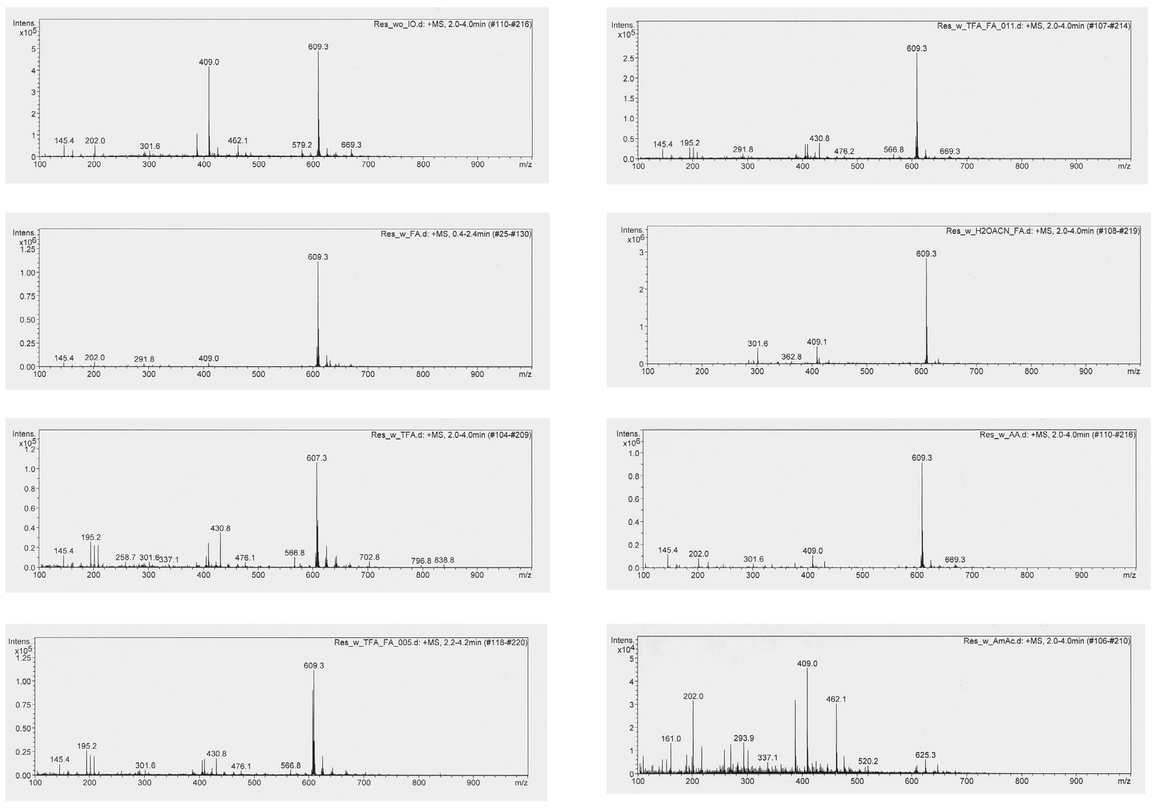

Fig. 4: ESI-Mass spectra of reserpine with different ionizer additives. Instrument / conditions: reserpine (500 pg/µl), direct infusion (4µl/min), Esquire 3000plus (Bruker Daltonic, Bremen, Germany) equipped with electrospray interface.

Conclusion ..... Depending on the chemical nature of the analyte it is necessary in ESI-LC/MS to avoid or add specific ions to obtain valuable and sensitive results. The ion formation process can be disturbed, suppressed or boosted, mostly due to the different stability of ions under the conditions of the electrospray. In peptide analysis alkali ions must be avoided, whereas glycosides are favoured by the addition especially of sodium ions. They can be measured with a higher sensitivity in the presence of alkali ions. The normal ionization process however, is the protonation, which can be supported by addition of organic acids or mixtures of them with acetonitrile. An exception is TFA, which is very widely used in HPLC of peptides. TFA works as an suppressor, which at least has to be counteracted by addition of formic acid (FA).

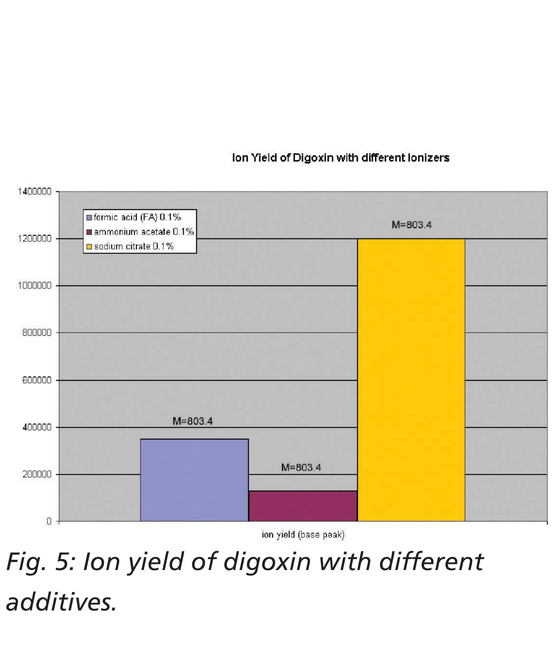

Fig. 5: Ion yield of digoxin with different additives.

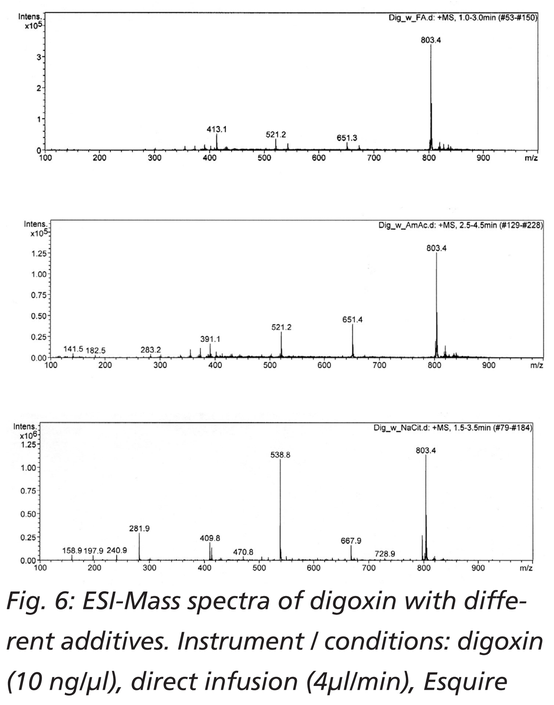

Fig. 6: ESI-Mass spectra of digoxin with different additives. Instrument / conditions: digoxin (10 ng/µl), direct infusion (4µl/min), Esquire 3000plus (Bruker Daltonic, Bremen, Germany) equipped with electrospray interface.

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