How to improve your ESI: Derivatization, Additives, Solvents and Blends as helpful aids to increase the sensitivity of analytes

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Summary

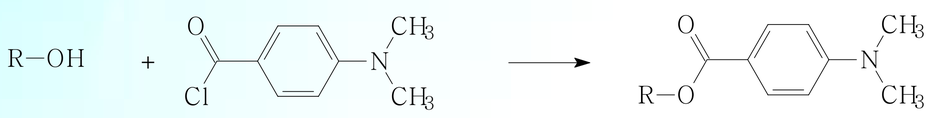
The model substances benzaldehyde and CP55940 (cannabinoid receptor agonist) and two available reagents are used to generate derivatives with improved sensitivity for ESI-LC/MS. Furthermore, the advantage of LC/MS additives and solvent blends is presented with different application techniques (pre-/post-column) to maximize the performance of an ESI source.

Motives

- Most LC/MS instruments are equipped with an electrospray (ESI) or a combined source (ESI/APCI). They allow the analysis of a wide range of substances and they provide a robust design.
- But there are some prominent substances that do not show any signals or just poor sensitivity with these ion sources - for example THC extracted from plasma (ESI pos.) or benzaldehyde (ESI pos./neg.) [1,2].
- The sample pre-treatment in general should be as easy and fast as possible. For this reason, the linkage of the MS label with the target molecule should not be a time consuming procedure.

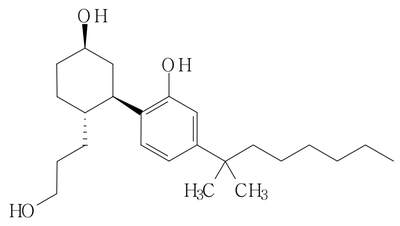
ESI(+) Label – Model systems for sensitivity enhancers

OH-Derivatives



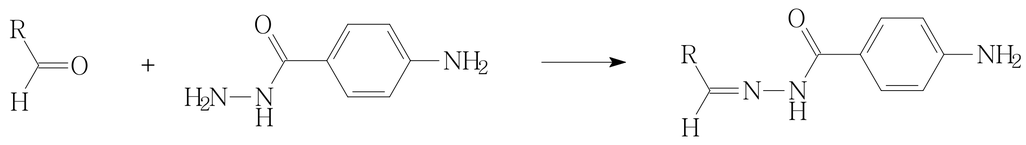
526118 4-(Dimethylamino)benzoyl chloride

Methods: 10 mg 4-(Dimethylamino) benzoyl chloride is added to 1 ml of a CP55940 solution (0.5 mg/ml in acetonitrile (34967 LC/MS grade)). The mixture is heated for 20 min at 55 °C. Mass spectra are obtained by direct infusion into the ESI source (4 µl/min).



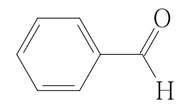
R-OH = C1112 CP55940

CHO-Derivatives



A41909 4-Aminobenzoyl hydrazide

Methods: 10 mg 4-Aminobenzoyl hydrazide is added to 1 ml of a benzaldehyde solution (0.5 mg/ml in acetonitrile (34967 LC/MS grade)). The mixture is heated for 20 min at 55 °C. Mass spectra are obtained by direct infusion into the ESI source (4 µl/min).



R = 09143 Benzaldehyde

Results: As expected, the reaction of CP55940 with the MS label results in two different derivatives, where one ([M+H]⁺=524.3 Da) or two OH residues ([M+H]⁺=671.4 Da) are linked with the labeling agent. The mass spectrum in fig. 1 shows the two major derivatives of CP55940; nearly the entire starting material is converted ([M+H]⁺=359.2 Da). The selectivity of the reaction is tested by varying the CP55940 concentrations. Fig. 1 (inset) shows the chromatogram peak-area of the masses 524 Da and 671 Da as a function of the CP55940 concentration. As a result, the selectivity of the reaction serves the needs for quantitative analysis.

Results: Benzaldehyde cannot be ionized by ESI (fig. 2, top). After the reaction only two major mass signals are found in the mass spectrum of the two reactants. Both refer to one single derivative ([M+H]⁺=240.0 Da, [M+Na]⁺=262.0 Da). 4-Aminobenzoyl hydrazide exhibits as a good derivatizing agent to provide the analysis of benzaldehyde with ESI.

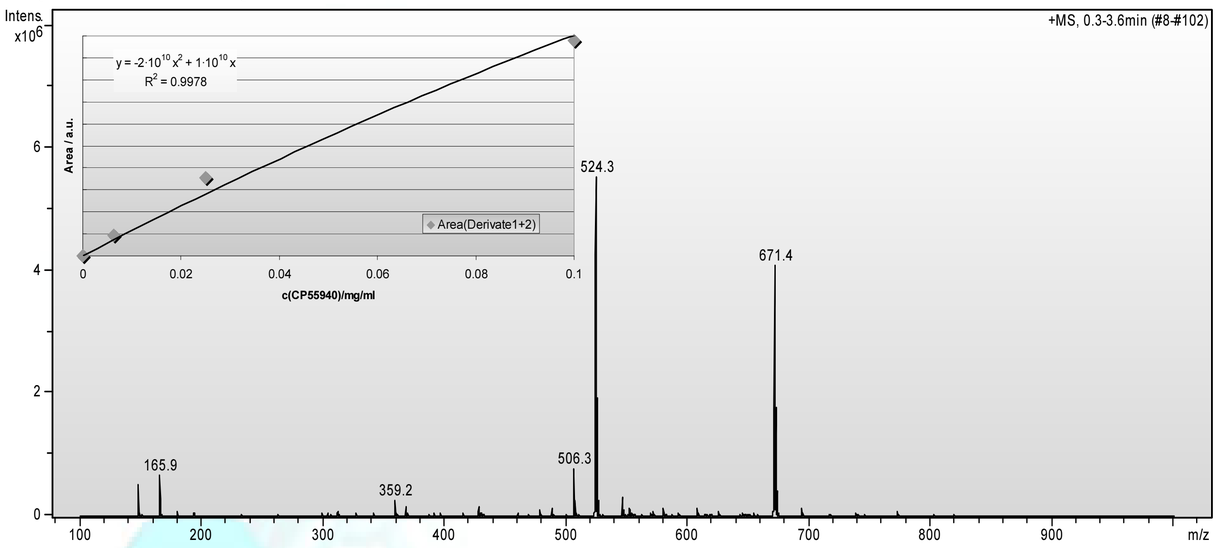


Fig. 1: Mass spectra of CP55940 after linkage with the MS label. One or two labels are attached to the OH residues resulting two major derivatives with [M+H]⁺=524.3 Da and [M+H]⁺=671.4 Da. The starting material (CP55950: [M+H]⁺= 377.2 Da, [M-OH]⁺= 359.2 Da) is almost completely converted.

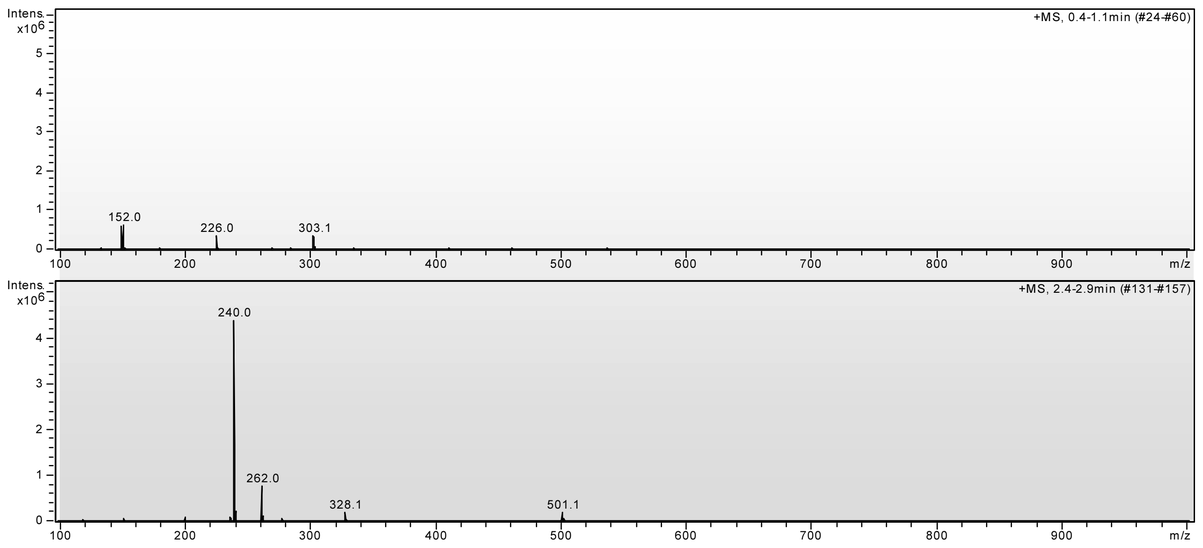


Fig. 2: Mass spectra of benzaldehyde (top) and its derivative (bottom). Benzaldehyde itself shows no signal (M=106.0 Da). After the reaction with the MS label, the derivative reveals a noticeable response ([M+H]⁺=240.0 Da, [M+Na]⁺=262.0 Da).

LC/MS Additives & Solvent Blends

Additives and premixed solvent blends represent the most common way to increase the sensitivity of an analyte. Often acidic compounds are used to enhance the ionization in pos. ESI mode. Fig. 3 shows the effect of different additives on the intensity of the reserpine signal (ESI pos., [M+H]⁺=609.3 Da). As expected acetonitrile, formic acid and water reveal as the best signal enhancers at a low pH. In some cases a high pH can positively effect the sensitivity of an analyte in pos. ESI mode as well (“reversed ionization”). This should be kept in mind, if one wants to optimize the HPLC at high pH. For example the analysis of opiates in plasma (fig. 4): The HPLC is performed with a gradient from 0.1 mM NH_4HCO_3 (pH=10) to methanol showing a good peak shape combined with short run times [3-7].

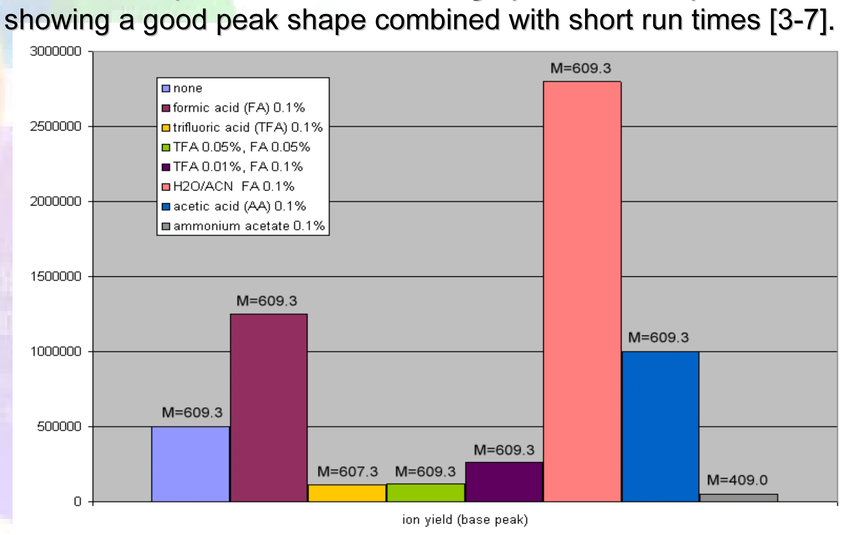


Fig. 3: Influence of the LC/MS additive on the ion yield of reserpine ([M+H]⁺=609.3 Da). Formic acid and acetonitrile reveal as best additives for this substance. Although an acid, TFA tends to ion pairing and suppresses the reserpine signal.

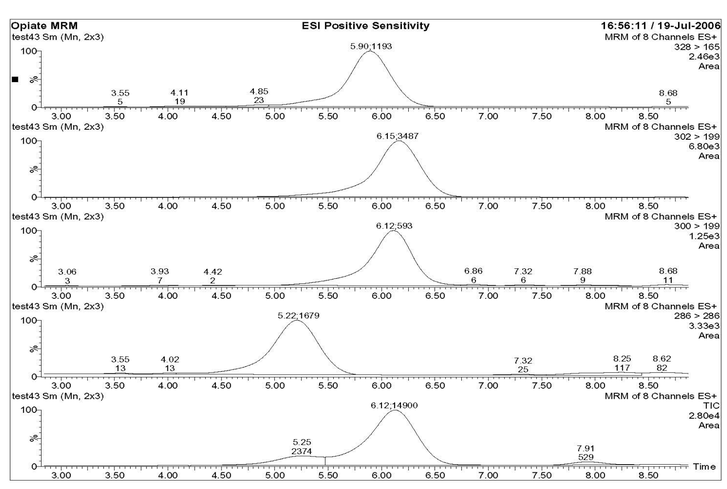


Fig. 4: MRM and TIC chromatograms of 4 different opiates (heroin, morphine, codeine, dihydrocodeine). The LC/MS setup consists of Waters 2695 Alliance system with a 2.1x50 mm column (C18, 3.5 µm) and a Quattro Micro API

References

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