LC–MSⁿ small-molecule drug analysis in human serum: could adducts have good prospects for therapeutic applications?

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Analyte adduct formation is well known in LC–MSⁿ [1,2]; however, in comparison to the most important means of ionization in ESI MS such as analyte protonation (positive ion mode) and deprotonation (negative ion mode), its application can be defined as rather limited. This is not really a surprise when considered that adduct formation was sidelined as a pitfall in LC–MSⁿ analysis equivalent to other sources of inaccuracy [3,4]. Nevertheless, the latest research demonstrated that this strategy of analyte ionization/fragmentation is a promising alternative for solving different problems concerning the analysis of small molecules relevant in clinical/forensic toxicology, with possibilities exceeding standard applications focused on higher sensitivities in comparison to protonation/deprotonation often observed and used for quantitative analysis [5,6]. In this article, different strategies of LC–MSⁿ analyses of problematic small-molecule drugs based on analyte adduct formation interesting for the field of bioanalysis were discussed on the basis of the latest literature. In focus are both small drugs with poor fragmentation, where the detection of small ion fragments is necessary when the conventional LC–MS² strategy is applied, and small drugs producing no stable ion fragments, where the product ion spectrum has no signals applicable for drug detection. Further, the application of analyte adducts in the LC–MSⁿ analysis of small-molecule drugs with problematic ionization based on protonation/deprotonation was also pointed out.

Generation of bigger ion fragments of small-molecule drugs with poor fragmentation for LC–MS² applications

To avoid the detection of small ion fragments, an analyte adduct formation/fragmentation strategy was developed for the generation of bigger ion fragments that could be used for LC–MS² analysis of γ -hydroxybutyrate (GHB), which was used as a model drug [7]. A GHB sodium acetate adduct ion was described as a target, of which the fragmentation was the basis for LC–MS² analysis to avoid the detection of ions smaller than 85 Da as described previous [8]. Sample preparation based on a protein precipitation performed with the mobile phase, together with a short analysis time of 3 min and a calculated LOD/LOQ of 1 μ g/ml, directly warranted a fast and sensitive analysis. Under conditions used with a mobile phase consisting of 10% A (H₂O/methanol = 95/5, vv) and 90% B (H₂O/methanol = 9/97, vv) both with 10 mM ammonium acetate and 0.1% acetic acid corresponding adducts were identified for GHB-D6 applied as internal standard for appropriate signal compensation.

Realization of LC–MS³ analyzes for small-molecule drugs with poor ion fragmentation

The same mobile phase conditions were used to develop a protein precipitation based sample preparation for GHB analysis, with a three-stage m/z separation strategy that was applied for drug detection by LC–MS³. The application of the GHB sodium acetate adduct ion enabled the optimization of a two-step fragmentation (185→103→85), which resulted in an LOD/LOQ of 0.28/0.96 μ g/ml [9]. It was demonstrated that the strategy of analyte adduct formation with the components of the mobile phase can be used successfully in the generation of two-step fragmentations in the case of small-molecule drugs that are unable to be analyzed with LC–MS³ by conventional analytical strategies [9].

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LC-MS² for the analysis of small-molecule drugs producing no stable ion fragments applicable for drug detection

An interesting challenge is the LC-MSⁿ analysis of small-molecule drugs that can pass conventional ESI ionization (like deprotonation), however, analyte ion fragmentation does not result in stable ion fragments applicable for mass spectrometric detection (the product ion spectrum of the deprotonated drug has no signals). Therefore, LC-MS² can be performed only by monitoring the deprotonated molecule (-ESI), such as in the case of valproic acid (VPA) (143→143) [10]. With further research, it could be demonstrated that analyte adduct formation/fragmentation may also be used for this problematic drug. The identification of VPA sodium acetate adduct ion and VPA acetic acid adduct ion made the LC-MS² VPA analysis on the basis of adduct fragmentation possible [11]. Again, sample preparation was performed by a simple protein precipitation and the LOD/LOQ of 0.45/1.0 µg/ml was appropriate for method application.

Application of LC-MS³ in the analysis of small-molecule drugs producing no stable ion fragments applicable for mass spectrometric detection

Further investigation of these problematic molecules, with VPA as a model drug, demonstrated that by the application of the analyte adduct with two molecules of sodium acetate (multiple adduct) the generation of a two-step fragmentation (307→225→143) is possible [12]. The method developed was characterized by an LOD/LOQ of 0.31/1.0 µg/ml and used successfully for the analysis of real samples. On the basis of these results it could not only be demonstrated that small-molecule drugs which do not produce stable ion fragments can be analyzed with LC-MS³ but also that multiple analyte adducts are applicable for quantification.

Analysis of small-molecule drugs with problematic ionization

In general, the application of LC-MSⁿ analysis is dependent on the possibility of molecule ionization. Problematic molecule ionization is an important reason to avoid LC-MSⁿ and apply different techniques, for example GC. An example of a substance relevant in clinical/forensic toxicology with problematic conventional ESI ionization is ethylene glycol (EG), which is usually analyzed with different GC-based methods with appropriate derivatization [13]. Besides the problematic ESI ionization, the EG molecular mass of 62 Da is another exclusion criterion for LC-MSⁿ that has been circumvented in the past only by EG derivatization preceding LC-MS/MS analysis [14]. However, a different method of EG LC-MS² analysis without the application of analyte derivatization was developed on the basis of adduct formation/fragmentation [15]. The identification of corresponding adduct ions with sodium and sodium acetate for EG and EG-D4 (internal standard) eliminated both the problem of analyte ionization (+ESI) and the problem of small-molecule drug analysis. In comparison to a problematic and time-consuming derivatization step, sample preparation based again on a simple protein precipitation. The calculated LOD/LOQ was 35/98 µg/ml. However, on the basis of the signal to noise ratio method application was recommended at a limit of 300 µg/ml.

Conclusion

It should be pointed out that the process of analyte adduct formation is an interesting possibility to solve various analytical problems concerning LC-MSⁿ analysis, including, but not limited to, method sensitivity [16]. So far, it has been demonstrated that controlled analyte adduct formation is an interesting alternative (and sometimes even the only effective possibility) for the LC-MSⁿ quantification of relevant small-molecule drugs [7,9,11–12,15]. Although it was already demonstrated that analyte adducts can be observed for numerous drugs with appropriate molecular structure and therefore the possibilities for its application can be defined as wide, analyte adduct formation seems to be applicable only if the conventional strategy of LC-MSⁿ analysis does not bring the expected results [17]. Adequate method sensitivity can be reached for different quantifications in combination with simple sample preparation strategies based on fast protein precipitation. Thus, the application of adduct formation/fragmentation is possible for the purposes of clinical/forensic toxicology after appropriate method optimization/validation. However, it should be taken into account that adduct formation is suggested to be strongly system-dependent, and that changeable analytical conditions are also an important factor when this strategy is applied [18,19]. Therefore, isotopically labeled analytes applied as internal standard are required for appropriate signal compensation. Since appropriate drug detection is essential when LC-MS/MS is applied in the bioanalysis, new analytical strategies involving analyte adducts and solving various analytical problems can also be expected in the future.

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