NITROGEN-CONTAINING CYCLIC COMPOUNDS AS DERIVATIZATION REAGENTS IN TANDEM MASS SPECTROMETRY

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Liquid chromatography – tandem mass spectrometry is one of the most powerful tools for determination of trace amounts of biological compounds. In comparison with scanning mass spectrometry measurements, a very high signal-to-noise ratio is achieved with selected reaction monitoring (SRM) detection, because the two levels of mass selection with narrow mass windows result in a very effective reduction in chemical noise [1]. As a result, an expected increase in sensitivity of one to two orders of magnitude over conventional mass spectrometry based approaches can be achieved [2]. While the first and the third quadrupoles act as mass filters to select a molecular ion of the analyte and a specific fragment ion of the analyte, the second quadrupole acts as collision cell where fragmentation takes place [1]. Since some compounds cannot be fragmented successfully because of their specific structure or the desired dissociation pathway giving the product ion of interest is only one of many dissociation pathways (thus limiting the abundance of desired product ion) [3], sensitive SRM detection of these analytes requires a specific derivatization.

In this work we present a comparison of five nitrogen-containing cyclic compounds as derivatization reagents for tandem mass spectrometric analysis of amino group-containing analytes. Five carboxylic acids were prepared by the reactions of commercially available starting materials (pyrrolidine, piperidine, 2,6-dimethylpiperidine, 1-methylpiperazine and morpholine) with bromoacetic acid as described elsewhere [4]. The carboxyl group of synthesized derivatization reagent was activated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide/N-hydroxysuccinimide (EDC/NHS) followed by covalent attachment of the model analyte tryptamine by its primary amino group, thus forming an amide bond [5]. Reversed phase ultra-performance liquid chromatography (RP-UPLC) with gradient elution was used for separation of derivatized tryptamine and mass spectrometric detection was performed in both SRM and selected ion monitoring (SIM) modes. During SRM, amide bond cleaves and a proton remains associated with the basic nitrogen of derivatization reagent moiety while the carbonyl group undergoes neutral loss [6]. As a result, the product ion is produced. The release and stability of these product ions during collision induced dissociation process are of critical importance for the detection sensitivity and were investigated by increasing collision energy and measuring peak areas of corresponding product ions in SRM mode, while SIM mode was required for the normalization of SRM data of all five different product ions originated from five different molecular ions.

Our results demonstrate that the yields of corresponding product ions generated from pyrrolidine, morpholine and piperidine moieties are similar and they are significantly higher in comparison with the yields of corresponding product ions generated from 2,6-dimethylpiperidine and 1-methylpiperazine moieties. However, the total amount of product ion depends not only on the fragmentation process, but also on the molecular ion yield in an electrospray ionization (ESI) source. Since basic groups can be ionized easily during positive ESI and 1-methylpiperazine moiety contains more basic nitrogen atoms than any other moiety investigated, the highest molecular ion yield could be expected. Taking this into account, an additional experiment was performed in order to compare the effects of morpholine and 1-methylpiperazine moieties on positive ESI of derivatized tryptamine. RP-UPLC with isocratic elution was performed in order to assure the same ionization conditions for both derivatized compounds. EDC/NHS strategy could result in a variety of side products [7]. Since baseline separation of desired derivatization products was not achieved, active NHS esters of (4-methyl-1-piperazinyl)acetic acid and 4-morpholinylacetic acid were synthesized as described elsewhere [4]. Because NHS esters react with primary amino groups, the products of interest were the same as synthesized employing EDC/NHS strategy, but no ultraviolet (UV) absorbing side products were determined suggesting that all consumed tryptamine was converted to the desired products. Taking this into account, the areas of UV peaks were used for SIM and SRM data normalization. Surprisingly, morpholine moiety demonstrated higher ESI efficiency.

Considering both ionization of derivatized tryptamine and fragmentation of molecular ions processes, approximately two times higher product ion yield was obtained using morpholine moiety in comparison with 1-methylpiperazine moiety. Consequently, morpholine moiety was shown being a promising product ion source for the SRM detection of analytes which cannot be fragmented successfully without derivatization.

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