Feasibility of Liquid Chromatography-tandem Mass Spectrometry Quantification of BAPN

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Introduction

This study deals with the feasibility of Liquid Chromatography-tandem Mass Spectrometry quantification of a prospective adjuvant molecule for cancer therapy, 3-aminopropionitrile commonly called BAPN. Initial tests showed that this small molecule improves treatment efficacy. A highly sensitive liquid-chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of BAPN in human plasma. A selective and sensitive quantitative method to determine 3-aminopropionitrile (BAPN) from human plasma aliquot has been developed with future validation in mind.

Experimental

Development of an analytical protocol

After Dansyl chloride derivatization, the sample was purified by solid phase extraction methods with Oasis® MAX 1cc (30 mg) extraction cartridges. The derivate molecule, Dns-BAPN was analyzed in LC-MS/MS and injected into a tandem mass spectrometer after formation of negative ions with electrospray ionization (ESI). Regarding the chromatography, a gradient HPLC method with a Hypersyl GOLD C18 stationary column (100x2.1 mm, particle dimension: 3µm) was employed. A first mobile phase consisted of 0.1% (v/v) acid acetic in water, and the second mobile phase composed of 0.1% acid acetic in acetonitrile. The flow was set at 200 µL/min. Concerning the mass spectrometer, the ESI source was operated in a negative mode. Double mass selectivity permitted the analysis of trace in complex matrices, like plasma. The selected reaction monitoring (SRM) mode was used with transition m/z 302/249 for the BAPN and m/z 342/249 for the FBAL-[$^2H_3$], the internal standard.

Method development

This method was developed thanks to the protocol of fluoro-beta-Alamine (FBAL). Indeed, the chemical structure of the target molecule has structural similarities with the previously studied molecule. Carboxyl Function (COOH) of FBAL was replaced by a nitrile (CN) in BAPN. FBAL is a metabolite generated by 5-fluorouracil (5-FU), an antitumor. The ultimate goal was to routinely dose the molecule of interest in human plasma. Each pre-analytical stage was studied to develop an analytical method for the routine assay of BAPN. This involved developing the preparatory phase samples by creating optimal conditions for each step (derivation, extraction/purification), in order to prepare a calibration range. The conditions for HPLC analysis and mass were developed. Once experimental parameters were fixed, a procedure was elaborated by taking into account each of the optimal experimental conditions. This permitted the traceability and reproducibility of the method. The final objective was to determine whether the dosage of the molecule of interest was possible in HPLC-MS/MS.

Results

The results were about the development of the method of derivatization. The advantage of this step was to add a dansyl chemical group, which permitted BAPN and FBAL to be separate by HPLC on reverse stationary phase C18. The mass spectrometry showed if the drug's derivatization was effective and permitted the optimal conditions of fragmentation to be selected, such as setting the collision energy or the ionization mode (positive or negative).

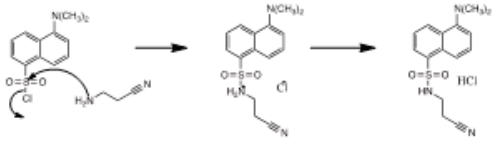

fig.1. *Reaction of BAPN with dansyl chloride.*

The negative ionization mode was chosen because it leads to the most simple fragmentation spectrum. Only one fragment ion product comes from fragmentation in negative mode compared to three ions product fragments for the positive mode. The derived FBAL, Dns-FBAL* (Mw=343 Da) had the same mechanism of fragmentation for both ionization modes.

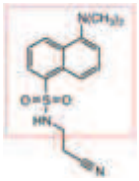

fig.2. *Ion Product Fragment (Mw=249) of Dns-BAPN in negative mode.*

BAPN and FBAL* solubilized in human plasma were incubated with dansyl chloride (10 mg/L) in acetone and sodium bicarbonate (buffer solution: 100mM; pH 11) at 60°C for 20 min. Plasma proteins have been previously precipitated by ammonium sulfate. Following addition of 0.5 mL water, the mixture was vortex mixed during 5 min, and centrifugated at 3500 xg for 10 min. The supernatant could be directly analysed by LC-MS/MS without performing purification on Oasis® extraction cartridges.

Conclusion

The developed protocol showed calibration curves with good linearity. However, these curves presented a large variability including, in profiles and ratio values of each range point. This lack of reproducibility didn't allow the method to be validated at this stage of development. Consequently, the protocol should be adjusted in order to obtain reproducible ranges. After that, the validation process could be reconsidered. The detection limit of quantification and detection must be determined.

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