Poster

[P27-7] P27-7: Assay

Chair: Wei-Chi Ku, Taiwan

Wed. Sep 27, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

(Wed. Sep 27, 2017 12:30 PM - 1:30 PM Annex Hall)

[P27-7-2] Toxicological screening for over 1000 compounds in whole blood samples using LC-MS/MS in MRM spectrum mode

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Keywords: LC-MS/MS, Screening, Toxicology

Background

Toxicological screening in the routine clinical environment focusses on panels of compounds based on the expected sample type being analysed. In clinical toxicology there is a growing need to enhance the capability in routine monitoring programs by increasing the number of compounds measured in a single analysis and at the same time deliver the highest confidence in compound identification to reduce false reporting. In this work, a conventional MRM method developed for routine clinical analysis (typically monitoring 2-3 MRM transitions for each compound) has been modified and extended to include a higher number of MRM transitions specific to each target (typically 6 fragment ions per-compound are monitored) resulting in higher confidence in compound identification. This paper describes the screening for over 1000 compounds using two methods in series by ultra-fast acquisition.

Methods

Whole blood was prepared by QuEChERS with inclusion of internal standard for quality control purposes. Analysis was performed by reversed phase UHPLC separation (Nexera LC, Shimadzu Corporation using a Restek Biphenyl 2.7um 2.1x100mm column). MRM Spectrum mode typically measured over 6 MRM transitions simultaneously throughout the entire analysis period using MRM peak intensity to create an MRM spectrum (LCMS-8060, Shimadzu Corporation).

Results

To evaluate the method, previous patient samples with known targets were used in assessing the viability of this approach. Target compounds were correctly identified including buprenorphine, nordiazepam, oxazepam, and temazepam with high matching scores (a similarity index score greater than 95 and a retention time window of 0.2mins). Despite the higher number of fragment ions monitored in MRM Spectrum mode, there was good agreement in signal intensity, linearity or reproducibility compared to conventional 2-3 MRM methods. MRM Spectrum mode enabled high data quality in quantitation by acquiring a high number of fragment ion transitions, each detected using an optimized collision energy. Each target compound had a corresponding optimized fragmentation spectra which may be used in routine library searching and compound verification using reference library match scores.

Conclusions

General unknown screening performed by MRM Spectrum mode for library identification using ultra-fast dwell time and pause time acquisition may be viable technique for toxicological screening programs.