Technical Note # TN-38

Fast and reliable Reaction Yield and Compound Purity Assessment in Synthesis Quality Control

Introduction

Typical quality control tasks in the synthesis, process development or production of chemical compounds are purity checks, the improvement of reaction yields or the characterization of contaminants and unwanted side products. Technologies commonly used for such QC tasks are HPLC with UV-based reference chromatograms or single quadrupole mass spectrometers for a simple mass assignment of compounds. In many cases, the results are quite ambiguous or difficult to interpret. However, applying other techniques with better performance normally requires larger investments. Speed of analysis is becoming increasingly important, particularly in industrial applications. Fast chromatography, coupled with a fast, reliable, high-performance MS/MS system is therefore highly desirable.

The amaZon SL uses innovative trap technology based on Bruker’s leading amaZon series, and delivers class-leading analytical performance. With high sensitivity – due to its proprietary dual ion funnel technology – and fast data acquisition at superior combinations of scan speed and mass resolution, it delivers uncompromised LC/MS^n data quality and flexibility, yet is affordable for any analytical lab. It is the ideal upgrade from single quad based LC/MS systems to enhance analytical capabilities in synthetic chemistry.

It is designed for easy, automated calculation of compound yield and purity, measurement of regulatory action levels of impurities as well as identification of target compounds. Easy-to-use OpenAccess software provides walk-up access for multiple users.

Results

The MS^n capability of the amaZon SL extends the information content of compound analysis far beyond a simple molecular weight assignment. Single fragmentation pathways in various MS^n steps allow observation of subsequent losses of side chains, enabling verification or even de-novo elucidation of molecular structures.

Fig. 1 shows the MS^n characterization of digitonin, a steroid glycoside. The complete glycan structure, down to the initial sugar unit, can be directly elucidated from the consecutive MS^n steps.

MS^n also helps in the confident verification of the identity of compounds from organic syntheses, process development, etc. The flexible API sources of the amaZon SL allows injection of solutions, e.g. from HPLC, as well as the direct analysis of solid samples using a direct insertion probe (DIP). The DIP provides for the straightforward analysis of samples without any tedious sample preparation. The example in Fig. 2 shows the DIP analysis of a synthetic compound.
After uptake of the solid sample from a vial, the glass capillary is inserted into the APCI source. Heating the capillary evaporates the sample into the source chamber where it is ionized by APCI. MS3 provided unambiguous identification of the synthetic compound. MS2 revealed only non-specific water loss and was therefore not sufficient for an unambiguous identification.

Compounds can be easily screened by comparing them with reference spectra stored in MSn libraries. Fig. 3 illustrates quick and reliable identification of natural compounds from myxobacteria by library search. High sensitivity and highly reproducible MS/MS data allow the direct comparison of the experimental data with library reference spectra.

The fast polarity switching leads to the detection of compounds in both ionization modes and ensures that no compound is missed. Fig. 3 shows the unambiguous identification of compounds such as Myxochelin A (detected in negative ion mode) and Myxalamid C (detected in positive ion mode). The total UHPLC runtime was only 15 min. Each UHPLC peak has a width of only 1-2 sec. Nevertheless, the high data acquisition speed of the amaZon SL allows polarity switching even in combination with MS/MS. In this case, fragmentation in auto-MS/MS mode was controlled by the SmartFrag™ algorithm. Here, the excitation amplitude for each compound is ramped up until the optimum dissociation energy is reached. This ensures optimum MSn spectrum quality and is the basis for highly reproducible MSn data that are ideal for use in reference library searches. High-quality, reproducible data are vital for obtaining high scores in library searches and for reliable, confident identifications.

OpenAccess software enables the amaZon SL to be used as an LC/MS walk-up solution that even MS beginners can use without special training. It also allows the use of the amaZon SL in a multiuser environment. The example in Fig. 4 shows a typical chemical synthesis quality control workflow where the presence of certain compounds must be verified. The user logs into the GUI and chooses a method from a fixed list. For the verification workflow, only the input of the expected elemental composition is required. The sample is then queued into the analysis batch, and as soon as the analysis is complete, the user receives the result via email as a PDF report. In this report – along with a base peak and extracted ion chromatogram – the intensity, mass accuracy and chromatographic purity of the expected compound are listed.

![Elucidation of the complete glycan structure](image)

Fig. 1: MSn experiment on digitonin, revealing the detailed structure of this steroid glycoside.

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![Identification of a synthetic compound](image)

Fig. 2: DIP (DirectProbe) analysis of a synthetic compound. MSn verifies the identity and correct structure of the synthesized analyte.
Large numbers of samples can be easily acquired this way. The visual representation of a 96-well plate shows the success rate of every sample by color-coding. In this example, the level of purity is reflected by a “traffic-light” color scheme.

Conclusions

The amaZon SL is the robust all-purpose answer to a wide variety of chemical, environmental, metabolic or forensic challenges. It enables rapid and sensitive identification, characterization and structural confirmation of compounds. The exceptional value of the amaZon SL is reflected by:

- Increased sensitivity – less compound is required to get an answer
- Measurement of a wider variety of compounds – thanks to increased data quality and information content
- Unambiguous answers and verification of molecular structures – from the combination of MS and MS/MS at remarkable mass accuracy
- Increased throughput in library searches – due to exceptional speed of MS and MS/MS, compatibility with any UHPLC system and fast polarity switching
- Operation in Expert or Open Access Environments – easy-to-use, robust and reliable setup
- Complete LC integration within Compass™ software – predefined LC/MS methods and simplified, automated post-processing tools.
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Keywords
- Compound identification
- Structural elucidation
- Quantitation
- Organic synthesis control
- Yield and purity control
- Solid sample analysis
- Multuser environment
- Open Access walk-up solution

Instrumentation & Software
- amaZon SL
- DirectProbe assembly
- Bruker APCI II ion source

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