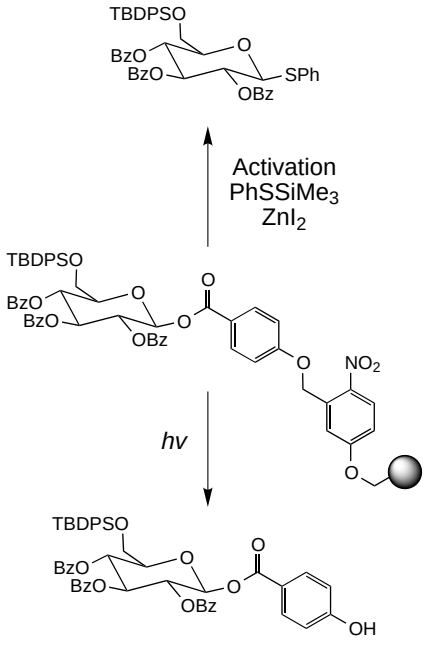

prepared on Merrifield resin attached to a photolabile linker. A spacer group between the anomeric centre and the linker allows a final mild cleavage of the products with retention of anomeric stereochemistry. However, intermediates can also be prepared on resin and cleaved with concomitant activation with $PhSSiMe_3$ to generate thioglycoside intermediates used for further elaboration of resin-bound oligosaccharides. This chemistry has been used in the synthesis of a branched dodecasaccharide related to the phytoalexin elicitor family.

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Simplified characterization of combinatorial libraries

While high-throughput screening and combinatorial chemistry are high-throughput solutions to previously rate-limiting steps, other lead optimization techniques remain labour intensive and of low throughput, placing a tremendous strain on discovery chemistry resources. Because of the sheer number of hits, the characterization of combinatorial libraries is difficult and sometimes tedious. Robust analytical methods that can provide rapid and simple determination of purity, identity and quantity of a synthesized compound are essential to ensure a rapid transition of lead compounds into development.

Until recently accurate quantification of the active component in a sample has not been possible, however, the advent of a novel nitrogen-specific HPLC detector enables the generation of exact potency measurements for any nitrogen-containing compound. Used in tandem with LC/MS, the new chemiluminescence nitrogen detector (CLND) can effectively and efficiently quantitate and characterize complex libraries and samples.

This profile focuses on one such solution – the Antek Model 8060 Nitrogen Specific HPLC Detector, an equimolar nitrogen HPLC detector, which provides true equimolar response for all nitrogen-containing samples allowing a single standard to quantitate multiple and complex samples without the need to re-calibrate. The instrument utilizes the accuracy and precision of its proven Pyro-chemiluminescence® technology to deliver equimolar response for all nitrogen-bearing compounds. Chemiluminescence is the clean, fast, interference-free method for determining all bound nitrogen in a variety of phases. The sample for analysis is injected directly from the HPLC system into the CLND where it is completely combusted in the presence of oxygen. Nitrogen from the sample forms nitric oxide, which is subsequently reacted with ozone to form nitric dioxide in its excited state. Once this species returns to ground state, the release of a photon is detected with a photomultiplier tube (Fig. 1). The measured intensity of chemiluminescence is directly proportional to the nitrogen present.

When characterizing crude products of a synthesis, methods based on traditional detectors, such as UV, can be misleading and time-consuming as the detector response is dependent on absorbance and the chromophores used and also requires purified references for every compound of interest. For detection by UV, the compound must contain a chromophore (Fig. 2). The Model 8060-CLND does not discriminate between compounds on this basis – it gives an equimolar response to nitrogen only, eliminating the need for derivatization and allowing quantitation of all nitrogen-containing compounds. It is the only HPLC detector capable of quantitating an unknown without having to use that unknown as a standard.

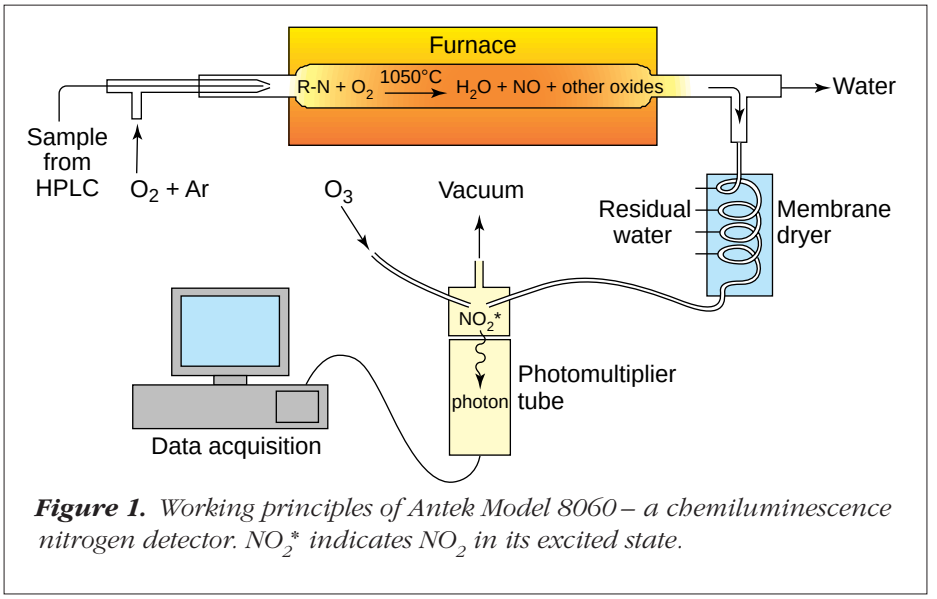

Figure 1. *Working principles of Antek Model 8060 – a chemiluminescence nitrogen detector. NO_2^* indicates NO_2 in its excited state.*

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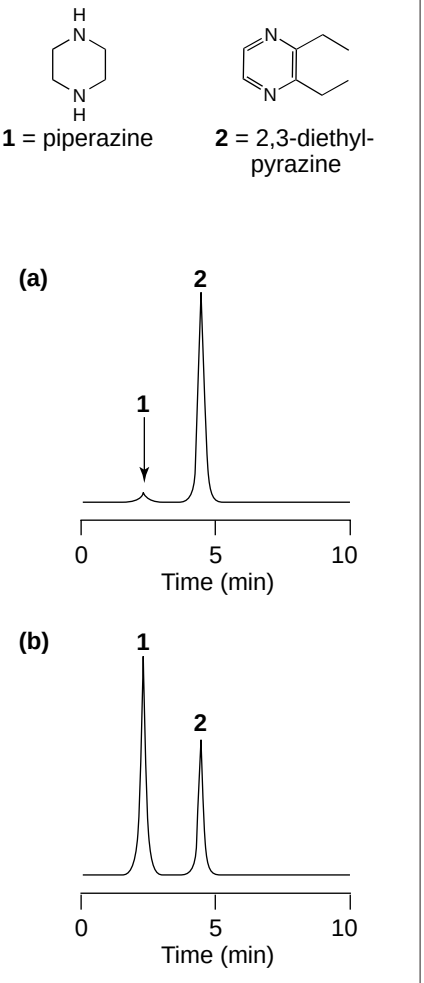

Figure 2. *Analysis of crude synthetic products by UV requires the presence of a chromophore, but with a chemiluminescence nitrogen detector (CLND) all nitrogen-containing compounds can be evaluated without the need for derivatization. The chromatograms of compounds* ***1*** *and* ***2*** *are: (a) UV at 254 nm and (b) CLND.*

Pharmaceutical compounds

To demonstrate the equimolarity and stability of analysis using this instrument, various pharmaceutical compounds were tested against acetaminophen as an external standard. Table 1 shows that accurate analytical results were obtained.

Table 1. Elemental analysis of varoius pharmaceutical compounds[a]

Compounds	Theoretical proportion of nitrogen (%)	Nitrogen found (%)
Acetominophen	9.27	
Procainamide	15.46	15.47[b]
Procainamide	15.46	15.45[c]
Theophylline	31.10	31.05[b]
Theophylline	31.10	31.16[c]
Thiamine	16.61	16.64
N-acetyl procainamide	13.39	13.40
Ephedrine	8.48	8.49

[a]All quantitation calculated on acetominophen as external standard; 20 μl injected per assay.
[b]First 10 injections of 50.
[c]Last 10 injections of 50.

The accurate quantification achieved enables analysts to calculate potency of compounds of interest using only a single, high-purity nitrogen calibration standard. The exact mass of the active compound within a synthesis yield can be determined, so that products from various formulations can be precisely quantified to establish their true comparative potency. Such advantages will increase the speed of characterizing chemical libraries and thus help to alleviate this rate-limiting step.

The Antek Model 8060 gives a linear response over a wide range of concentrations, providing nitrogen detection with equivalent accuracy from 1 ng to 100 mg. It has a sensitivity threshold <0.3 ng of nitrogen (<10 picomoles) enabling detection of limited quantities of sample.

This powerful new tool has a wide range of applications in laboratories where the ability to detect and quantify nitrogen will enhance and simplify experimental procedures. Its applications include combinatorial chemistry, pharmaceutical, biomedical, biotechnical, agricultural, foods and petrochemical industries. Within pharmaceutical applications, this instrument allows the accurate quantitation of product yields and potency measurements to give a true ranking order of hit compounds for lead selection in drug discovery programmes. It should prove a valuable addition to the pharmaceutical analytical laboratory where it will speed up lead optimization and the transition of new compounds to product development. The detector is also an ideal enhancement for structure–function studies, synthetic peptide areas and even DNA mapping.

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