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Liquid chromatography–mass spectrometry with chemiluminescent nitrogen detection for on-line quantitative analysis of compound collections: advantages and limitations

David Corens*, Michel Carpentier, Marc Schroven, Lieven Meerpoel

Synthesis Enabling Technology Group, Department of Medicinal Chemistry, Johnson and Johnson Pharmaceutical Research and Development, a Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

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Abstract

Recently, alternative detection methods such as chemiluminescent nitrogen detection (CLND) have been coupled successfully with HPLC for quantification. This detector produces a signal proportional to the number of moles nitrogen present in the compound. Sample concentration of compounds with a known formula can be determined by use of an external calibration standard such as caffeine. Hence, the CLND can be used without the need for primary standards of the compound with unknown concentration, which enables the use of this detector for high-throughput analysis. In this work, the reliability and pitfalls of this coupled LC–MS/CLND are demonstrated. Nitrogen detection is specific as it only gives a response for nitrogen containing compounds and universal since it only gives a linear response. Nevertheless the lower response for N=N and N–N containing compounds has been evaluated in this study.

Keywords: Chemiluminescent nitrogen detection; Quantitative analysis; LC–MS

1. Introduction

The use of combinatorial and automated synthetic methods have enhanced the drug discovery process in the pharmaceutical industry. Consequently, the analyst has been confronted with a huge amount of samples requiring analysis in a short period of time. Previously, most compounds were synthesized in larger amounts and identified by NMR, elemental analysis and mass spectrometry. In order to meet the requirements for high-throughput analysis liquid chromatography (LC) coupled with mass spectrometry is still playing a leading role in molecular weight confirmation of synthetic materials [1–5].

Purity determination in LC–MS are mostly limited to a qualitative purity usually determined by UV-detectors. Although many analytical laboratories still utilize one wavelength UV-detector - generally 214 nm or 254 nm - it is rather preferable to use a diode array detector (DAD) to have a better "overall" UV-purity. Nevertheless purity determinations need to be interpreted carefully. Currently, different other detection methods, such as evaporative light scattering detection (ELSD) [6–8], can also be implemented and have already been evaluated for measuring the purity of compound libraries. These library compounds produced by parallel synthesis are mostly obtained in small quantities. Reaction workup with liquid–liquid extraction or solid-phase extraction often deliver compounds with a purity of <90%. In most of the cases the compounds are further purified with reversed phase HPLC to yield compounds with a high purity (>95%). These small quantities, from a few milligrams to hundred milligrams, can still contain solvents or water from incomplete evaporation or drying. These impurities will elute by RP-HPLC but in most of the cases are undetected by ELSD as they are too volatile, are undetected by UV as they mostly do not contain chromophores and even are undetected by CLND as they do not contain nitrogen. Relative purity analysis can cause serious prob-

* Corresponding author. Fax: +32 14 605344.
E-mail address: dcorens@prdbe.jnj.com (D. Corens).

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lems since even high purity but low quantity of a compound can contain invisible impurities undetectable by UV and/or ELSD.

Quantitative analysis by use of a chemiluminescent nitrogen detector (CLND) can be beneficial as it will determine the actual purity of compounds instead of the relative purity determined by UV. ELSD can be used for actual purity as the response of the ELSD is independent of compound structure and responds to the amount of material. Unfortunately different calibration curves are needed for different compound classes.

Chemiluminescent nitrogen detection has demonstrated to be useful in pharmaceutical analysis e.g. for the determination of underivatized amino acids [9], to quantify metabolites [10], proteins [11], for peptide analysis [12], in environmental analysis e.g. for analysis of contaminated groundwater [13] and waste water [14], and in the food industry [15] e.g. for the determination of caffeine content in beverages [16]. The advantages of coupling this detector with gas chromatography (GC) [17,18], supercritical fluid chromatography (SFC) [19,20] and liquid chromatography [21] has been clearly investigated in the past. The use of CLND within combinatorial chemistry has first been discussed by Fitch et al. [22] and later by Taylor et al. [23]. In this paper the advantages and disadavantages of CLND within high-throughput analysis is investigated.

2. Experimental

2.1. Chemicals and reagents

Formic acid and methanol (Uvasol) were provided by Merck (Darmstadt, Germany). Buffer was prepared in water purified with a Milli-Q system (Millipore, Bedford, MA, USA). Test compounds were obtained from Acros Organics (New Jersey, USA) and in-house synthesized compounds.

2.2. Instrumentation

2.2.1. Liquid chromatography/mass spectrometry (LC/MS)

The LC–MS system consisted of an Alliance 2795 HT (Waters, Milford, MA, USA) liquid chromatograph connected to a ZQ quadrupole (Micromass, Manchester, UK) mass spectrometer equipped with an orthogonal Z-electrospray interface and controlled by MassLynx software. The needle voltage used was 3.5 kV (positive ionisation mode). The cone voltage was 10 V. Nitrogen gas was used as nebulizing gas. Ten microliters of sample were injected on column. The source was maintained at 140 °C. A Xterra HPLC column 4.6 mm × 100 mm, 3.5 um (Waters, Milford, MA, USA) was used. A standard gradient was applied: solvent A was formic acid (0.1%) and solvent B was methanol. The gradient was programmed from 100% A to 5% A in 13.5 min to 100% B in 1 min. Column temperature was maintained at 40 °C and a flow rate of 1.5 ml/min was applied.

2.2.2. Chemiluminescent nitrogen detector

The Antek Instruments CLND 8060 (Houston, TX, USA) was used. Combustion furnace was set at 1050 °C, inlet oxygen flow at 250 mL/min and inlet helium flow at 50 mL/min and ozone 25 ml/min.

3. Results and discussion

The aim of this study was to evaluate the advantages and disadvantages of this nitrogen specific detector in combination with LC–MS. Initially the detector characteristics were explored by use of a diversity of compounds. Previously, the major problem with CLND coupled with LC was the nebulization. Additionally water must be eliminated from the system before entering the reaction chamber with ozone. Nowadays the new type of nebulizer barrel allows a more efficient sample introduction into the pyrolysis tube.

The use of the direct flow injection MS to identify molecular weight together with direct injection CLND technique for concentration determination for high-throughput quality control, has already been published a few years ago [24]. Unfortunately, flow injection has its drawbacks mainly caused by co-elution problems. A good separation method is needed to ensure the reliability of the results obtained. Obviously liquid chromatography is restricted to nitrogen free mobile phases. Nevertheless the use of flow injection mass spectrometry coupled with direct injection CLND is only applicable for highly purified compounds. The combination of liquid chromatography with CLND is preferred in the case of many side reaction products, impurities and low concentrations.

The only chromatographic limitation with LC–MS/CLND is the restriction in the use of nitrogen free mobile phase solvents [22,23].

3.1. Mobile phase

The CLND was configured within an existing LC–MS equipment. This has the advantage of using both MS, UV and Nitrogen detection in one experiment. Thus qualitative analysis (purity profiling with LC, MS, UV) can be performed in combination with quantitative analysis (concentration determination with CLND). The signal of CLND is directly related to the number of moles nitrogen present independent of the structure except for nitrogen (N_2) and N=N containing molecules and all mobile phase systems must be nitrogen-free in order to keep the noise to a minimum. Obviously, the use of this detector is limited to nitrogen containing compounds. Fortunately most of the drug-like molecules contain nitrogen which makes CLND an ideal technique in drug discovery. However in our standard generic gradient methods ammonium acetate and acetonitrile were used and needed to be replaced by a nitrogen free alternative. Initially,

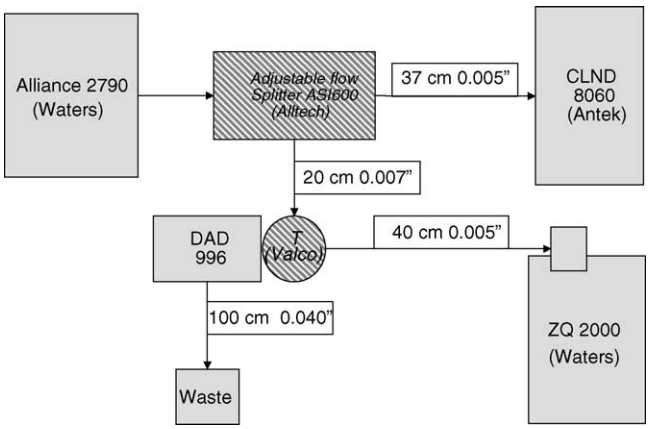

Fig. 1. Schematic flow of LC–MS/DAD/CLND setup.

it was essential to clean the whole LC-system. Solvent channels used for acetonitrile and ammonium acetate could not be used anymore even after an intensive cleaning and had to be replaced since minor nitrogen contaminant disturbed the CLND signal. Several new gradient methods have been evaluated and finally a method was developed with formic acid 0.1% and methanol as the mobile phase solvents.

3.2. Splitter

One of the biggest problems encountered in the early days of the coupling of the CLND with HPLC was the reproducibility mainly caused by clogging of splitter [25] and nebulizer. In our setup different splitters such as low dead volume T-splitters (Valco), accurate splitters (LC packings) and variable flow splitters (Alltech) have been evaluated. In most of the cases a low dead volume T-splitter (Valco) is used for LC–MS/DAD setup. This setup was also evaluated with the CLND detector by use of two T-splitters, one for DAD and one for CLND. However an effect on the CLND signal was observed since gradient conditions were used. Because of a change in viscosity of the mobile phase composition during the gradient run, the flow splitted to the detector will change during one run. This effect will have an impact on the

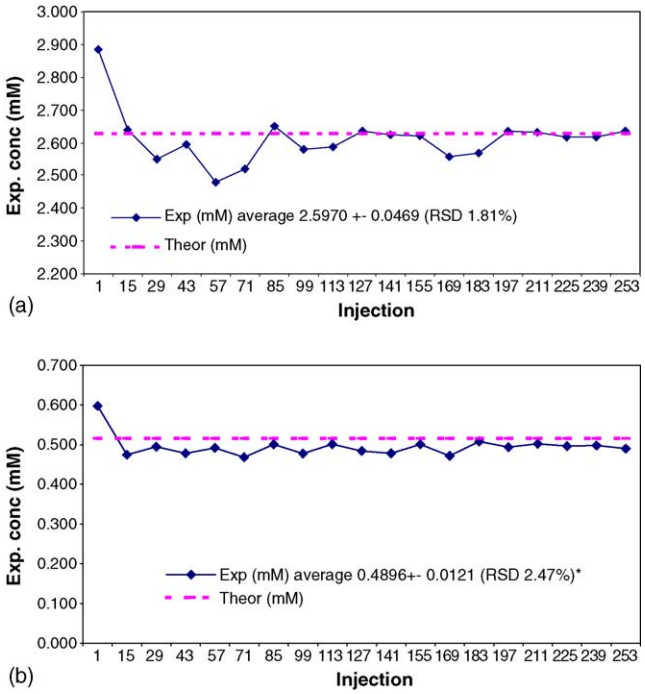

Fig. 3. CLND reproducibility with caffeine.

CLND-response. As the signal is quantitative it was essential to use an accurate splitter or variable flow splitter instead of a T-splitter to have a constant exact flow to the CLND detector. Finally, a variable flow splitter has been implemented and the tubing length has been optimized. Split ratio can change when clogging occurs in the tubing after the splitter. The use of an optimal diameter and short length tubing is recommended. Fig. 1 shows the setup of the splitters together with the tubing length which is crucial to obtain reproducible and reliable results especially for analyzing low soluble compounds.

The change in mobile phase composition during gradient elution has no effect on the intensity of the CLND signal and no shift in peak area was observed. Comparison of a small set of frequently used solvents (DMSO, MeOH and H_2O) shows that the response is not influenced by the solvent.

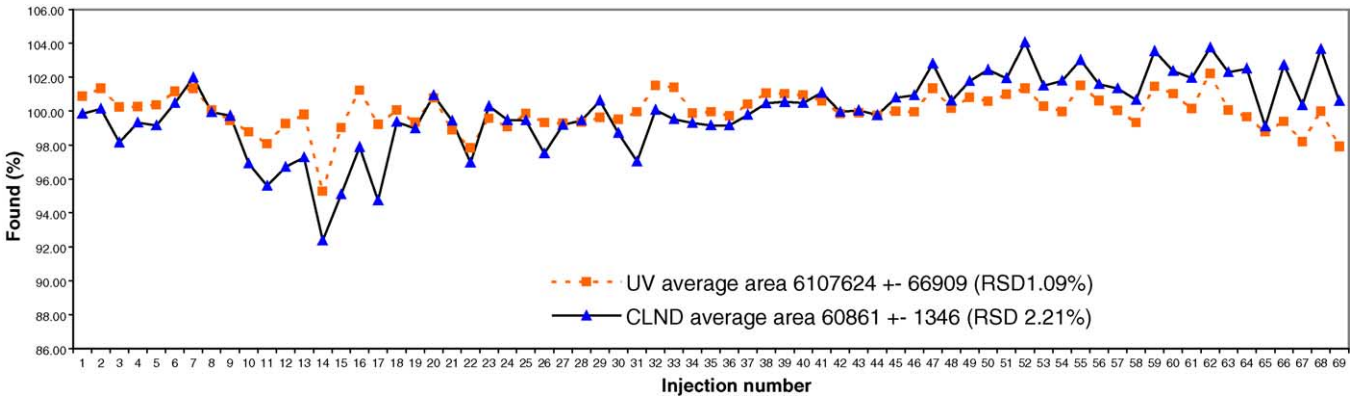

Fig. 2. Comparing reproducibility of UV and CLND signal response.

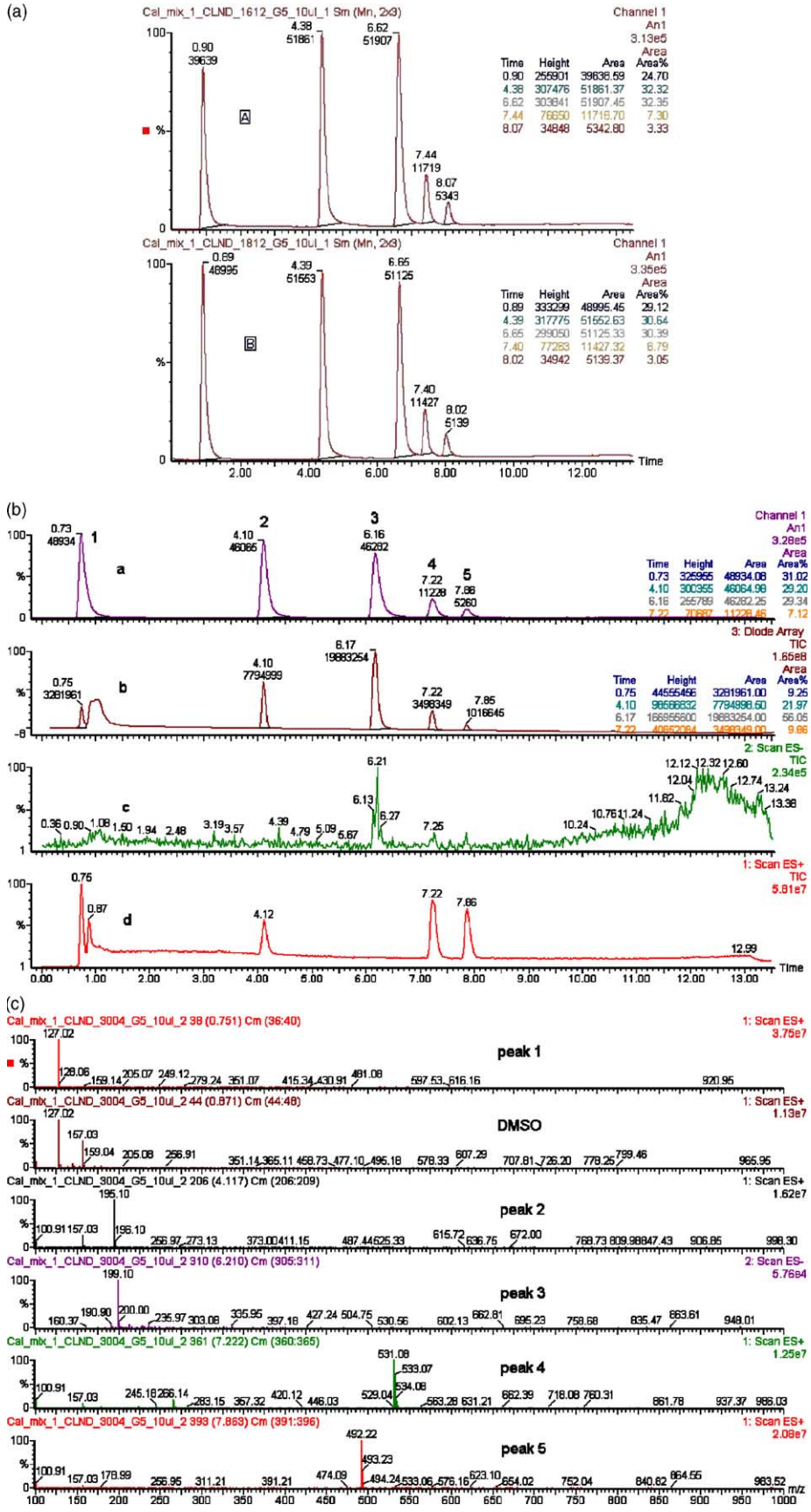

Fig. 4. (a) Quality control test mixture. (b) LC–MS/DAD/CLND TIC of test mixture. (c) Mass spectra of test mixture.

3.3. Limit of detection

The limit of detection (LOD), defined as the signal to noise ratio of 3:1, was tested on a compound containing one nitrogen (JNJ34645AAA - Haloperidol). However, there is a difference in LOD between a flow injection analysis and an on-column analysis. This limit of detection for on-column analysis was determined as 3 ng nitrogen on column.

3.4. Reproducibility

The reproducibility of injection is very important in quantitative analysis. Fig. 2 shows the signal response of the CLND detector in combination with the response of the diode array detector normalized to 100% response for the average response. The relative standard deviation (RSD) on the average UV peak area is 1.09%. The RSD on the average CLND-response is 2.21%.

Fig. 2 shows a comparable trend of the response for both detectors. Once there will be a difference in this trendline for UV compared with CLND, problems with the flow to the UV or CLND, as a result of clogging of tubing or splitter problems, can directly be observed with the flow to the UV or CLND. Definitely the reproducibility of LC-injection is the most critical parameter influencing the detector response.

In order to check the reproducibility of CLND a sample of 0.5 mM and 2.6 mM caffeine was injected during a set of 253 samples from different structure classes and concentrations. This test allows to determine whether the calibration curve needs to be optimized or re-measured. Fig. 3a and b shows the fluctuations of the response, expressed in concentration units (mM) from the theoretical expected concentration of a 0.5 mM and 2.6 mM caffeine solution with a RSD of respectively of 1.8% and 2.47%. (excluding the first point which was measured before equilibration of the detector). The flat line shows the expected concentration. Our experience has learned that the calibration curve is stable for more then one week continuous use. Nevertheless the use of a control sample of caffeine within a sample set is advisable.

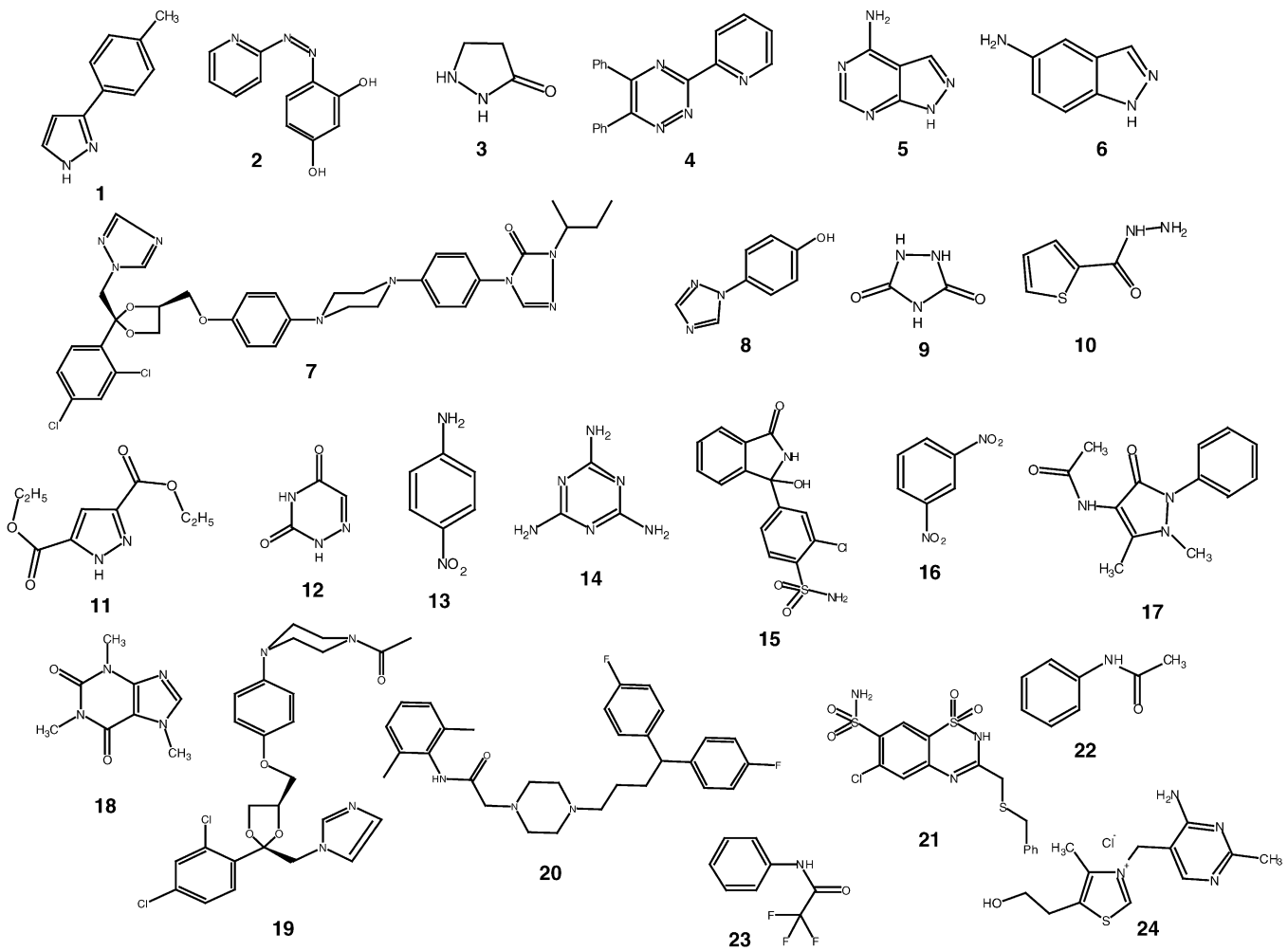

Fig. 5. Structures of compounds selected for equimolarity study.

3.5. Clogging problems

The problems of clogging of the nebulizer was critical in the previous version of the nebulizer barrel leading to a cooking of the product in the beginning of the furnace [26]. Besides the flow to the CLND, which was adjusted to 80 μL/min, the spray of the nebulizer is the most crucial parameter that need to be adjusted. This spray will enter the pyrolysis tube. It is essential to have a good aerosol that passes the inlet without having contact with the pyrolysis tube The initial part of the nebulizer has a lower temperature (>150 °C) then the second part (>400 °C). In particular this will have the greatest effect on the reproducibility for less soluble products. A disturbance from the ideal spray of the nebulizer was experienced for those low soluble products in a non-optimized spray.

Thus quality control of the system is essential and the best way is to have a low soluble compound in the test mixture. If the response of this compound decreases over a time period, this will be an indication of an abnormal spray at the nebulizer tip.

Our quality control sample consists of a test mixture of five compounds (in order of elution: compounds **14**, **18**, **16**, **19** and **20** shown in Fig. 4) with different nitrogen content, different selectivity and different solubility. As shown in Fig. 4a the equimolar response of the three first peaks in the chromatogram B shows the reliability of the detector signal. In chromatogram A of Fig. 4a, a distortion is observed in the first peak. This peak is coming from melamine, a compound with low solubility under gradient elution. This phenomena can be caused by a bad nebulizer spray, clogging in the tubing or problems with the filling of the pyrotube. Compared with the UV-signal the peaks of the CLND have more tailing and are often broader. This can have an influence on the automated integration of peaks. Fig. 4b shows the different TIC-chromatograms for the different detectors used in the LC–MS/DAD/CLND setup. A stronger tailing effect can be observed with the CLND signal compared with the DAD-signal. Fig. 4c shows the mass spectra for the five compounds used for the quality control.

3.6. Equimolarity of response

The major disadvantage of the CLND detector at this moment is the equimolarity of response for N=N containing compounds. These compounds have a tendency to yield N_2 on combustion in stead of nitrogen oxide. This has an effect on the CLND signal because N_2 does not react with ozone during the chemiluminescent reaction and a less then equimolar response is observed. For those type of compounds the area will be not reliable and the concentration will be underestimated.

A set of representative structures with various number of nitrogen and different types of N=N bonds was selected to validate the equimolarity of the CLND instrument (Fig. 5).

These compounds were first analysed by elemental analysis to determine the absolute purity. For CLND-analysis compounds were dissolved in 100% DMSO and different

Table 1
Summary table of equimolarity test

Compound	Formula	Theor. (mM)	Exp. (mM)	Found element analysis (%)	Calcd. no. of nitrogens	Average found CLND (%)	STDEV (%) (n = 6)	No. of nitrogens found	No. of nitrogens for N—N or N=N
1	$C_{10}H_{10}N_2$	3.534	0.724	102	2	20.50	1.38	0.4	0.4
2	$C_{11}H_9N_3O_2$	2.317	1.105	100	3	47.67	1.86	1.4	0.4
3	$C_3H_6N_2O$	2.288	0.290	72	2	12.67	0.82	0.4	0.4
4	$C_{20}H_{14}N_4$	1.032	0.646	101	4	62.67	2.42	2.5	0.5
5	$C_5H_5N_5$	2.324	1.708	101	5	73.50	3.27	3.7	0.7
6	$C_7H_7N_3$	3.884	2.434	110	3	62.67	1.63	1.9	0.9
7	$C_{35}H_{38}Cl_2N_8O_4$	0.762	0.564	101	8	74.00	1.41	5.9	0.95
8	$C_8H_7N_3O$	3.142	2.189	100	3	69.67	3.08	2.1	1.1
9	$C_2H_3N_3O_2$	2.556	1.197	103	3	46.83	2.32	1.4	0.4
10	$C_5H_6N_2OS$	3.873	2.808	101	2	72.50	4.18	1.5	1.5
11	$C_9H_{12}N_2O_4$	2.436	1.868	101	2	76.67	3.72	1.5	1.5
12	$C_3H_3N_3O_2$	2.242	1.917	102	3	85.50	1.87	2.6	1.6
13	$C_6H_6N_2O_2$	7.652	7.372	101	2	96.00	4.68	1.9	
14	$C_3H_6N_6$	2.102	1.937	101	6	92.17	4.31	5.5	
15	$C_{14}H_{11}ClN_2O_4S$	1.515	1.424	97	2	94.00	3.69	1.9	
16	$C_6H_4N_2O_4$	2.954	2.974	98	2	100.67	1.21	2.0	
17	$C_{13}H_{15}N_3O_2$	2.050	1.971	99	3	96.17	6.31	2.9	
18	$C_8H_{10}N_4O_2$	0.532	0.520	102	4	97.67	2.58	3.9	
19	$C_{26}H_{28}Cl_2N_4O_4$	0.735	0.719	100	4	97.83	2.99	3.9	
20	$C_{30}H_{35}F_2N_3O$	0.510	0.473	100	3	92.83	5.49	2.8	
21	$C_{15}H_{14}ClN_3O_4S_3$	1.228	1.152	100	3	93.83	2.48	2.8	
22	C_8H_9NO	3.768	3.850	100	1	102.17	2.99	1.0	
23	$C_8H_6F_3NO$	5.393	5.285	100	1	98.00	2.83	1.0	
24	$C_{12}H_{17}ClN_4OS{\cdot}HCl$	3.048	2.909	98	4	95.43	8.50	3.9	

Table 2
Automated data processing in Excel

Sample name	MW found	RT	DAD purity (%)	Area CLND	Number of *N* in compound	Cone (mol/l)	Cone (mg/ml)	MW of compound	Amount product solved (mg/ml)	Theoretical concentration (mM)	% found
JNJ_6236750_AAA_2910	X	9.08	97.34	74934.13	3	2.01E-02	6.6541	331.0854		2.00E-02	101
JNJ_6236997_AAA_2310	X	7.53	100	74557.81	3	2.00E-02	6.4728	323.1845		2.00E-02	100
JNJ_27052519_AAA_2910	X	7.3	98.24	78779.28	3	2.11E-02	5.9459	281.154		2.00E-02	106
JNJ_27052532_AAA_2910	X	5.96	100	73324.77	3	1.97E-02	4.9084	249.1477		2.00E-02	99
JNJ_27052545_AAA_2310	X	6.76	38.43	72599.42	3	1.95E-02	5.2115	267.1383		2.00E-02	98
JNJ_27052558_AAA_2910	X	8.14	98.48	69151.02	3	1.86E-02	5.2640	283.1088		2.00E-02	93
JNJ_27052584_AAA_2910	X	7	100	70908.27	3	1.91E-02	5.0158	263.1634		2.00E-02	95
JNJ_27052597_AAA_2910	X	7.56	98.66	71954.13	3	1.93E-02	5.4745	283.1088		2.00E-02	97
Caf 0.51 mg ml 2910 G5 H_2O 5 ul 1	X	3.85	100	10971.38	4	2.37E-03	0.4593	194.0804	0.51	2.63E-03	90
JNJ_27052610_AAA_2910	X	7.82	97.93	77522.84	3	2.08E-02	6.8077	327.0582		2.00E-02	104
JNJ_27052623_AAA_2910	X	8.21	98.57	73831.16	3	1.98E-02	6.2905	317.1351		2.00E-02	99
JNJ_27052636_AAA_2910	X	8.78	97.96	80513.95	3	2.16E-02	6.8514	317.0638		2.00E-02	108
JNJ_27052649_AAA_2910	X	8.37	34.55	73634.61	3	1.98E-02	6.2726	317.0698		2.00E-02	99
JNJ_27052675_AAA_2910	X	6.56	98.35	75254.01	3	2.02E-02	6.2492	309.1689		2.00E-02	101
JNJ_27052688_AAA_2910	X	8.32	88.83	81273.05	3	2.18E-02	6.9167	317.1351		2.00E-02	109
JNJ_27052701_AAA_2910	X	7.47	100	78063.7	3	2.10E-02	6.2277	297.1477		2.00E-02	105
JNJ_27052714_AAA_2910	X	8.02	100	73844.74	3	1.98E-02	6.2520	315.1383		2.00E-02	99
Caf 0.51 mg ml 2310 G5 H_2O 5 ul 2	X	3.87	100	10504.43	4	2.27E-03	0.4413	194.0804	0.51	2.63E-03	87
JNJ_27052727_AAA_2910	X	8.91	97.62	73266.35	3	1.97E-02	6.5180	331.1088		2.00E-02	98
JNJ_27052740_AAA_2310	X	8.21	100	77190.75	3	2.07E-02	6.4494	311.1634		2.00E-02	104
JNJ_27052766_AAA_2310	X	8.65	98.71	70082.4	3	1.88E-02	6.2383	331.1088		2.00E-02	94
JNJ_27052779_AAA_2910	X	8.88	100	71346.36	3	1.92E-02	7.1921	375.0582		2.00E-02	96
JNJ_27052792_AAA_2910	X	9.11	100	72849.77	3	1.96E-02	7.1475	365.1351		2.00E-02	98
JNJ_27052805_AAA_2910	X	2.66	33.61	0	3	out of linearity		365.0698		2.00E-02	
JNJ_27052818_AAA_2910	X	9.26	97.93	71424.15	3	1.92E-02	7.0081	365.0698		2.00E-02	96
JNJ_27052831_AAA_2910	X	7.68	98.71	69313.61	3	1.86E-02	6.6564	357.1689		2.00E-02	33
Caf 0.51 ma ml 2910 G5 H_2O 5 ul 3	X	3.86	100	11251.25	4	2.42E-03	0.4701	194.0804	0.51	2.63E-03	92
JNJ_27052857_AAA_2910		9.2	98.08	69577.98	3	1.87E-02	6.8305	365.1351		2.00E-02	94
JNJ_27052870_AAA_2910		4.12	90.22	54249.2	3	1.46E-02	3.2370	221.1164		2.00E-02	73
JNJ_27052883_AAA_2910		6.11	87.08	64625.89	3	174E-02	4.4365	255.0775		2.00E-02	87
JNJ_27052896_AAA_2910		6.46	84.76	65003.39	3	1.75E-02	5.2314	299.0269		2.00E-02	87
JNJ_27052909_AAA_2910		5.41	32.72	68150.42	3	183E-02	4.3095	235.1321		2.00E-02	92
JNJ_27052922_AAA_2910		6.41	88.96	72166.05	3	1.94E-02	5.7992	299.0269		2.00E-02	97
JNJ_27052948_AAA_2910		4.44	90.14	67795.43	3	1.82E-02	4.3598	239.107		2.00E-02	91
JNJ_27052961_AAA_2910		7	88.23	65516.63	3	1.76E-02	5.0966	289.1038		2.00E-02	88
Caf 0.51 mg ml 2310 G5 H_2O 5 ul 4	X	3.85	100	11292.48	4	2.43E-03	0.4717	194.0804	0.51	2.63E-03	92

concentration ranges and injections were investigated. Each measurement was repeated six times at different days and caffeine was used as external calibration standard. Table 1 shows the experimental measured concentration together with the expected theoretical concentration. From these results we could observe the lower response for N=N and N–N containing molecules. Surprisingly this non-equimolarity is inconsistent over a broad range of compounds (J&J property compounds are not included in Table 1). For example, the measured actual concentration for 3-(4-methylphenyl)-1*H*-pyrazole **1** is 20.50% of the expected concentration whereas for dimethyl 1*H*-pyrazole-3,5-dicarboxylate **11** the average found concentration in CLND is 76.67% of the expected concentration. Other J&J property substituted pyrazols show the same inconsistency. Surprisingly *N*-(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1*H*-pyrazol-4-yl)-acetamide **17** yields an experimental response of 96.17%. From these results we can conclude that no uniform re-calculation factor can be used for structurally related N–N containing compounds since the difference in response is depending on the nature of the N=N or N–N bond in the compound. Definitely, for these type of compounds it is better to use a separate standard calibration curve. Nevertheless, careful interpretation of the results is needed when dealing with N=N or N–N containing compounds.

3.7. Automation

The major pitfall with the automated triggering molecular weight and extracting area from the analog signal of the CLND, is the probability to have co-elution. This can be avoided by manually looking at the peaks in the Openlynx report. Nevertheless this is time-consuming. Although there is a trend to use fast gradients for LC–MS it is absolutely necessary to focus on well separated peaks for LC–MS/CLND to obtain reliable results in the concentration determination. Otherwise co-eluted peak would give rise to overestimated concentrations.

An automated process was implemented to calculate the unknowns concentration of compound collections. The calculation of the concentration were achieved by use of caffeine as external standard. This compound gives a good response and is easily soluble in H_2O. A set of serial diluted caffeine samples 0.01, 0.1, 0.5, 1 and 2 mg/ml was used for the calibration curve. The LC–MS vendor software Masslynx and Openlynx (Waters, Milford, MA, US) and Microsoft Excel 2000 and Visual Basic 6.0 were used to write in-house an Excel sheet for processing. An example is shown in Table 2 and contains expected concentration, purity based on DAD, area, retention time, triggered MW, experimental concentration and % found. The concentration was calculated by use of the calibration curve. The number of nitrogen atoms was extracted from the formula specified in the Openlynx sample report. The second column was used to check the triggered molecular weight. If no specified molecular weight is found because of the low MS-response, a manual check of the chromatogram and mass spectrum is necessary. Nevertheless all additional fields are filled based on the highest peak in DAD.

4. Conclusion

The use of chemiluminescent nitrogen detection in combination with LC–MS has shown to be applicable for combined qualitative and quantitative analysis. Nevertheless the CLND has not the same robustness as UV-detector or ELSD. Taking into account the problems that can occur with the splitter and nebulizer, this CLND offers a solution for time-efficient quantification of compound collections. The inconsistency in response for N=N containing compounds can be solved by use of separate specific standard calibration curve for each compound.

For libraries of compounds synthesized in small quantities it is difficult to determine the yield of reaction. Robotic systems are able to weigh small quantities but even for a pure product the error will be greater then a manual micro weighing process. As most of the products still contain impurities like water, salts, resin impurities, residual solvent, TFA or other inorganic impurities, the sample weight will be overestimated. Consequently the concentration of the solutions prepared by robotic systems will differentiate from the expected concentration. Additionally solubility problems in the solvent can also occur. Obviously it is important to determine the actual concentration of the plates delivered to the biologist or central stock unit since further dilution of master plates will multiply the error on the concentration of the daughter well-plates and led to misleading SAR-data. This concentration determination cannot be done by classical UV-based methods since not enough compound will be available to prepare standard solutions and will be too time-consuming.

Although, further improvements in robustness of CLND can be made and special attention need to be made towards N–N containing compounds, this detector brings additional value for high-throughput quantification.

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