Journal of Chromatography A, 1411 (2015) 63–68

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A charged aerosol detector/chemiluminescent nitrogen detector/liquid chromatography/mass spectrometry system for regular and fragment compound analysis in drug discovery

Yutao Jiang*, Daniel Hascall, Delia Li, Joseph H. Pease

Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form 28 July 2015
Accepted 31 July 2015
Available online 1 August 2015

Keywords:
Fragment
Chemiluminescent nitrogen detector
Charged aerosol detector
Pharmaceutical
Liquid chromatography
Purity

ABSTRACT

In this paper, we introduce a high throughput LCMS/UV/CAD/CLND system that improves upon previously reported systems by increasing both the quantitation accuracy and the range of compounds amenable to testing, in particular, low molecular weight "fragment" compounds. This system consists of a charged aerosol detector (CAD) and chemiluminescent nitrogen detector (CLND) added to a LCMS/UV system. Our results show that the addition of CAD and CLND to LCMS/UV is more reliable for concentration determination for a wider range of compounds than either detector alone. Our setup also allows for the parallel analysis of each sample by all four detectors and so does not significantly increase run time per sample.

1. Introduction

In pharmaceutical drug discovery, the rapid and accurate identification and quantitation of a wide variety of compounds (collectively termed quality control or QC) is critical in ensuring that data generated from subsequent experiments is reliable. Many different systems for carrying out this QC have been reported [1–4]. Unfortunately, there is not a single "universal" detector that is capable of accomplishing this task. Compounds range from small "fragments", with molecular weights <250 g/mol, to more typical compounds with molecular weights up to ~800 g/mol. Many detectors used in quantification, such as mass spectrometry (MS) or ultraviolet absorbance (UV), rely on measuring standard response curves for each compound of interest, which can be unreliable if the purity of the standard compound is inaccurate. Particularly challenging are the small fragment compounds, as these often do not ionize or absorb light readily making detection and quantitation by MS or UV difficult.

Structure identification of compounds is most commonly done by observing the parent molecular ion or accurate mass using mass spectrometry (MS) [5–7]. Separation systems such as liquid chromatography (LC) [8] or gas chromatography (GC) [9] are often coupled to a MS to improve selectivity of the target analyte. LCMS is able to detect over 95% of the compounds in our QC lab using electrospray ionization (ESI). For compounds which do not ionize readily under ESI conditions, such as volatile and fragment compounds, GCMS with electron impact (EI) ionization is used. For the select few compounds which are undetectable by either LCMS or GCMS, alternative methods such as NMR can be used [10,11].

Quantitation of compounds, namely the purity and concentration, are assessed by a wide array of methods [1–4]. A common method utilizes UV percent area under the curve (AUC) at a fixed wavelength, such as AUC at 254 nm [12]. A major limitation of this method is that most compounds differ in their molar absorptivity which makes comparison of UV peak areas of different compounds in a sample an unreliable way of assessing weight percent purity [12]. In a simple example, a 50:50 mixture by weight of two compounds where compound A has 3× the absorptivity of compound B at 254 nm would result in an apparent peak ratio A:B of 3:1, leading to the wrong conclusion that compound A is 75% pure by weight. Since the purity of A is actually 50% by weight, this miscalculated purity will cause the amount of compound A weighed out for subsequent testing to be lower than the target amount. Assessment of absolute purity and concentration of a sample is more reliably done by universal detection methods such as charged aerosol detection (CAD) or chemiluminescent nitrogen detector (CLND).

* Corresponding author.
E-mail address: yutaoj@gene.com (Y. Jiang).

http://dx.doi.org/10.1016/j.chroma.2015.07.112
0021-9673/

CAD enables determination of absolute weight percent purity by measuring the amount of surface charge on analyte particles [13]. CAD is typically used in conjunction with liquid chromatography (LC) and involves first nebulizing the LC column eluent with nitrogen gas. The smaller droplets are then selected for by an impactor and desolvated into particles in an evaporation chamber before being passed into a mixing chamber. There, a secondary flow of nitrogen gas that has been positively charged by passage through a high voltage generated corona is then mixed with the analyte particles to facilitate transfer of positive charge from the nitrogen to the analytes. The charged analyte particles accumulate in a collector where an electrometer produces a signal proportional to the amount of charge present. Because only the amount of surface charge is measured, differences in analyte chemical properties, such as ionizability or UV absorption, that limit other detectors, do not affect CAD response [14–16].

Despite its advantages, CAD's major setback is its inability to accurately quantify volatile compounds [13]. Because CAD can only measure analytes that form particles, it cannot detect compounds that are highly volatile or do not readily form particles. Another potential problem is that the compounds used to generate a standard curve must have similar particle-forming propensity as the analytes of interest. Analytes with a lower propensity to form particles than those in the standard, such as low molecular weight fragments, will generate a disproportionally low or absent signal resulting in the calculation of an inaccurately low concentration [13]. Conversely, if sample analytes form particles more readily than those in the standard, the resulting signal will be disproportionally greater, resulting in the calculation of an inaccurately high concentration. Changes in the mobile phase solvent composition can also alter method sensitivity by affecting the particle-forming ability of analytes so care must be taken to ensure that mobile phase ratio is isocratic prior to reaching the CAD [13]. CAD is generally the preferred detector for quantitation as it returns a peak area that is directly proportional to weight percent, but it is not reliable when assessing volatile compounds, typically those of MW <400 g/mol. The analyses of such compounds are better suited to CLND [17].

The chemiluminescent nitrogen detector (CLND) measures analyte concentration based on nitrogen content of the molecule. Similar to CAD, CLND is also compatible with HPLC [20]. CLND burns the sample in an oven to first form NO and then NO_2, which then goes into an ozone reaction chamber in order to excite an NO_2 electron [18]. When the electron returns to ground state, a photon is emitted and detected by a photomultiplier tube (PMT) to produce the signal. Because CLND does not require analytes to form particles, many volatile compounds unresponsive on CAD are readily detected by CLND. Since the configuration and number of nitrogens in the compound affects the signal response of the CLND, standards encompassing the various nitrogen configurations and content of the compounds to be analyzed must be run to determine an appropriate response factor for each configuration of nitrogen found in the molecules [18]. This response factor corrects for differences in CLND signal response due to variations in nitrogen content and arrangement. This poses a potential problem if CLND is to be used for purity determination as the structure and nitrogen content of the target compound is usually known, but structure is not known for impurities thus making quantification difficult. Furthermore, CLND is also unable to detect molecules lacking nitrogen.

Since many of the limitations of one detector could potentially be overcome by the others, we sought to utilize the strengths of four detectors without compromising efficiency by adding CAD and CLND to a standard LCMS/UV system. Along with the LCMS/UV data, CAD provided weight percent purity and analyzed compounds lacking nitrogen and undetectable by CLND, while CLND enabled quantitation of the more volatile compounds, such as low molecular weight fragment compounds. This system utilizes all four detectors for each compound which improves the accuracy and reliability of measured concentrations without significantly lengthening the run time per sample.

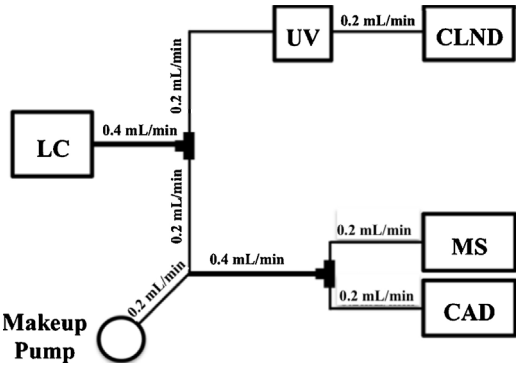

Fig. 1. Diagram of the LCMS/UV/CAD/CLND system. Flow rate from the LC was split 1:1 with one line leading to the UV then CLND and the other split between the MS and CAD. A make-up pump running a mobile phase gradient inverse to that of the LC was placed before the MS/CAD in order to maintain an isocratic gradient to the CAD.

2. Materials and methods

2.1. Solvents and standards

Flurbiprofen, ketoprofen, ibuprofen, theophylline, isoniazid, acetaminophen, dibucaine, methyl pyridazine-4-carboxylate, 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol, azobenzene were purchased from Sigma–Aldrich (St. Louis, Missouri, USA); 5-(p-tolyl)-1H-tetrazole and N,N′-diacetylhydrazine were purchased from TCI (Tokyo, Japan); 1-(tert-butyl)-3-methyl-1h-pyrazole-5-carbonyl chloride was purchased from Thermo Fisher Scientific (Loughborough, UK); ketoconazole was purchased from TRC (North York, ON, Canada); and reserpine was purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). All other compounds are proprietary to Genentech and synthesized in-house.

2.2. Instrumentation

The LCMS/UV/CAD/CLND system consisted of an LCMS/UV system (Shimadzu) with LC-30AD solvent pump, 2020 MS, Sil-30AC autosampler, SPD-M30A UV detector, CTO-20A column oven; a Corona Veo RS CAD (Thermo Scientific); model 8060 CLND (Antek). LabSolutions software (Shimadzu) was used for data collection and analysis.

2.3. Methods

A Waters BEH C18 column (30 mm × 2.1 mm, 1.7 μm) was used for all measurements. Mobile phase A was 0.1% formic acid (FA) in H_2O; mobile phase B was 0.1% FA in methanol (MeOH). LC flow rate was 0.4 ml/min. Fig. 1 diagrams the layout of the system. Flow rate from the LC was split 1:1 between the UV and CAD/MS and the CAD/MS flow was split 1:1 again between the CAD and MS. Since CAD response is sensitive to the mobile phase composition, a makeup pump (flow rate 0.2 ml/min) was placed between the LC and CAD/MS which ran an inverse gradient to the LC to maintain an isocratic A:B mobile phase ratio of 50:50 to the CAD/MS. The flow rate reaching all detectors was 0.2 ml/min. The following gradient was run on the LC system: 2% B, 0.0–0.2 min; 2–98% B, 0.2–2.1 min; 98% B, 2.1–2.9 min; 98–2% B, 2.9–3.0 min; and 2% B, 3.0–3.5 min.