Use of Charged Aerosol Detection as an Orthogonal Quantification Technique for Drug Metabolites in Safety Testing (MIST)

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ABSTRACT

The publication of the U.S. FDA guidelines in 2008 on drug metabolites in safety testing (MIST) requires that the relative quantitation of human metabolites be obtained as soon as feasible in the drug development process. The goal is to ensure that at least one of the animal models used is not only producing the same metabolites found in humans but that these metabolites are found in the same relative abundance. Identification of drug metabolites is typically accomplished using various LC-MS or LC-MS/MS approaches. However, metabolite quantification is often difficult to accomplish with MS techniques alone. In some cases the addition of UV detection is sufficient for metabolite quantification. However, this requires that both parent drug as well as its metabolites possess a sufficiently active chromophore. Unfortunately, this is not always the case. Charged aerosol detection can detect any nonvolatile, and many semivolatile compounds, typically with low-ng sensitivity. Furthermore, as response is similar for all compounds and independent of chemical structure, it is ideal for measurement drugs and metabolites.

Presented here are the results from two test cases used to evaluate the usefulness of charged aerosol detection for MIST: buspirone (which has a strong UV chromophore) and erythromycin (which has a weak UV chromophore). These drugs were analyzed directly and following incubation with human liver microsomes (drugs at ≤100 µM). The five major metabolites for buspirone and the four major metabolites for erythromycin were identified using an LTQ-Orbitrap MS and quantified by charged aerosol detection. The range, linearity, and sensitivity of this approach will be discussed as well as techniques for lower concentration levels. Charged aerosol detection is an important complementary technique to those already being employed in an industry capable of extending the range of in vitro drug metabolites that can be monitored during the drug development stage of testing.

INTRODUCTION

Interest in metabolite and trace impurity analysis by the pharmaceutical industry is intensifying due to concerns with mass balance studies, regulatory commitments in reporting API impurities, MIST, and cleaning validation of manufacturing equipment. Most often an analytical requirement for accurately reporting the level of metabolites or impurities is to obtain reference standards or use a radiolabeled drug approach. Because many of these standards are unavailable and the radiochemical approaches are time consuming and expensive, quantification of drug metabolites can often be difficult in early development stages. The situation is further exacerbated since several types of HPLC detectors, such as UV or evaporative light scattering detection (ELSD), either do not provide uniform response across the target analytes or lack the sensitivity to detect these compounds. Although electrospray ionization (ESI) MS techniques are extremely sensitive and powerful approaches, ionization efficiency can vary between metabolites and lead to quantification issues.

The Corona® Charged Aerosol Detector (CAD®) is mass sensitive and can be added to the traditional HPLC-UV or LC-MS platform. This detector provides the most consistent response across all nonvolatile and some semivolatile analytes of all HPLC detection techniques.[1] The detector works by charging particles, as shown in Figure 1, and is not dependent on light scattering which can vary between analytes. The work presented here examines the combination of UV and MS detection already employed in this field, with charged aerosol detection for quantification of drug metabolites in two specific cases. This work was completed in collaboration with Bristol-Myers Squibb (BMS), using a mix of HPLC equipment and method conditions.[2] Although one analytical system and condition is emphasized, a variety of analytical equipment was used throughout the study.

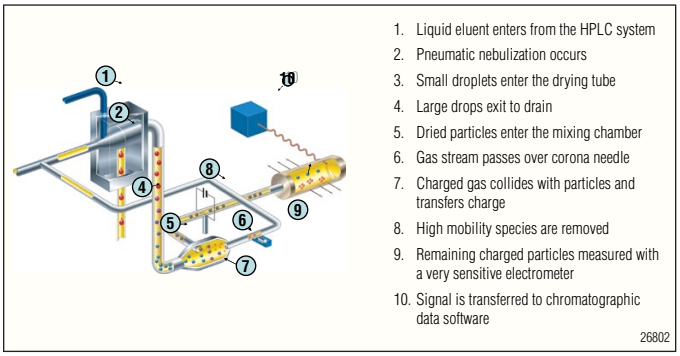

Figure 1. CAD flow path schematic.

METHODS

Sample Preparation

The buspirone and erythromycin standards were incubated at 1, 30, 60, and 100 µM substrate concentrations of human liver microsomes (HLM). At the end of the incubation period, the samples were quenched with an equal part of acetonitrile. The final analytical concentration of the metabolite samples discussed in this work is therefore one half of the concentration listed as the sample name (i.e., 60 µM pre-equals 30 µM postincubation).

Analytical Conditions

Column:	SB C18, 1.8 µm, 4.6 × 150 mm
Mobile Phases:	A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile
Flow Rate:	1 mL/min
Total Run Time:	15 min

Detection Conditions

UltiMate® 3000 Diode Array (Thermo PDA used in some examples):

UV Wavelength 1: 220 or 210 nm depending on example (values listed)

UV Wavelength 2: 254 nm

Corona *ultra*™:

Filter: High

Gas: 35 psi Nitrogen

Range: 100 pA full scale

LTQ Orbitrap High Resolution Mass Spectrometer (HRMS)

Full scan MS with 1 × DDS

Flow Split

Valco three-way splitter

Volumes fixed by backpressure of different tubing diameters and lengths

Volume measured at CAD inlet.

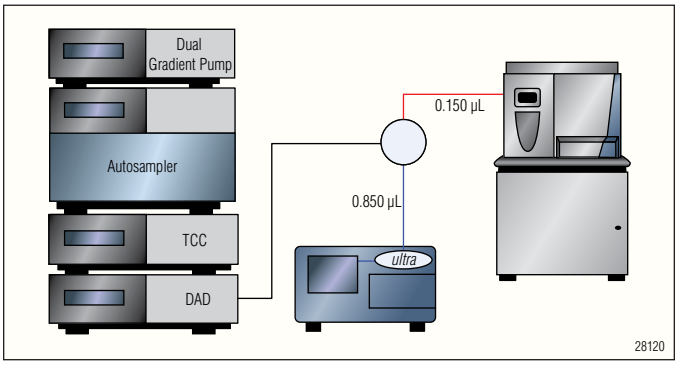

Figure 2. Schematic of one of the LC-CAD-MS systems used for analysis, with a total flow of 1 mL/min split ~5.7/1 to the CAD.

RESULTS

Buspirone

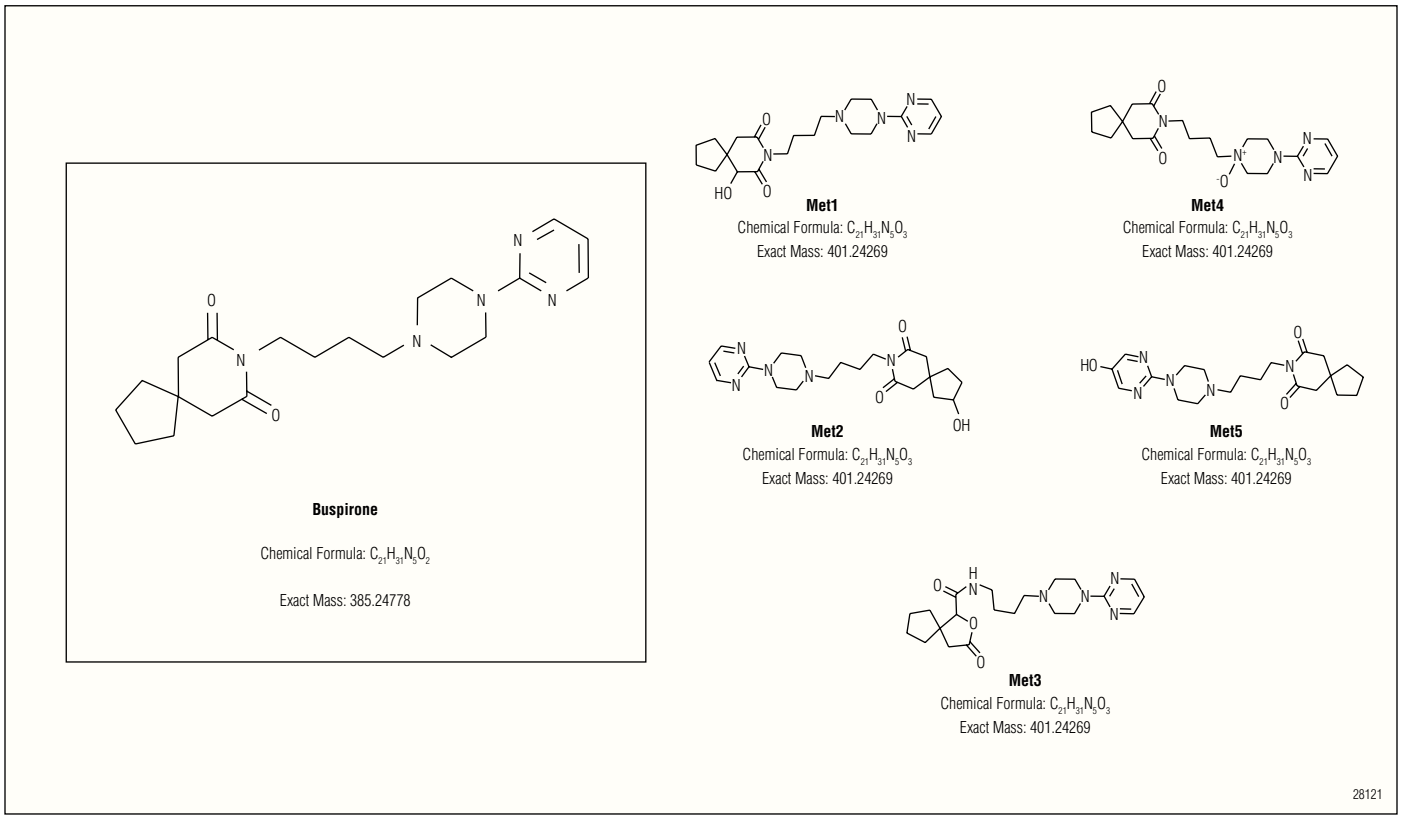

Figure 3. Structure for buspirone and five of its known metabolites.[3]

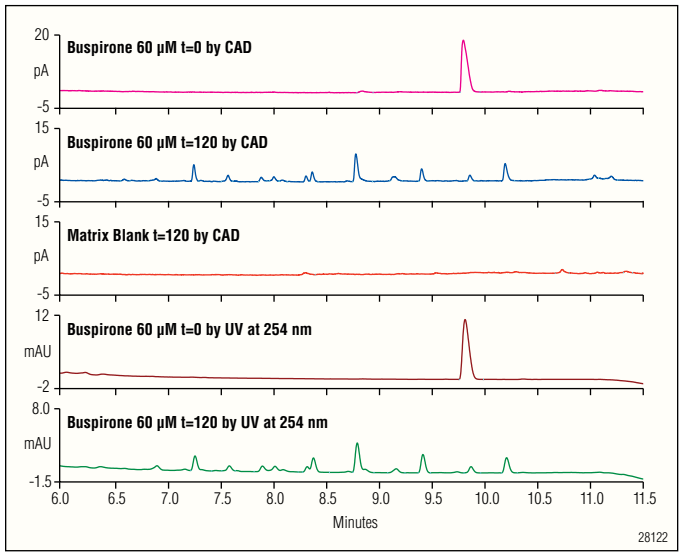

Figure 4. Chromatographic results for 60 µM buspirone before and after 120 min incubation in HLM.

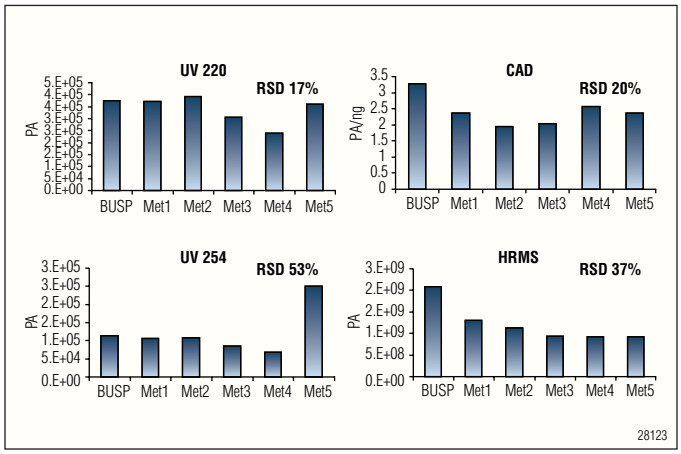

Figure 5. Response as peak area for injection of equal concentrations of buspirone and the five metabolites. Response deviation of each detection technique is calculated as percent RSD.

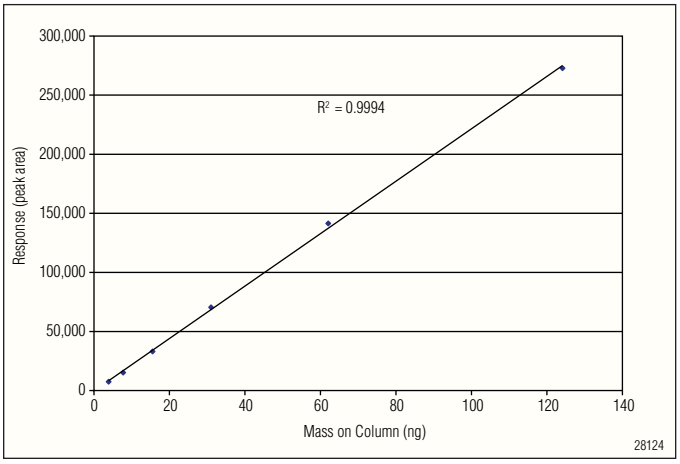

Figure 6. Linear response curve for buspirone with CAD from ~4 to 124 ng on column.

Erythromycin

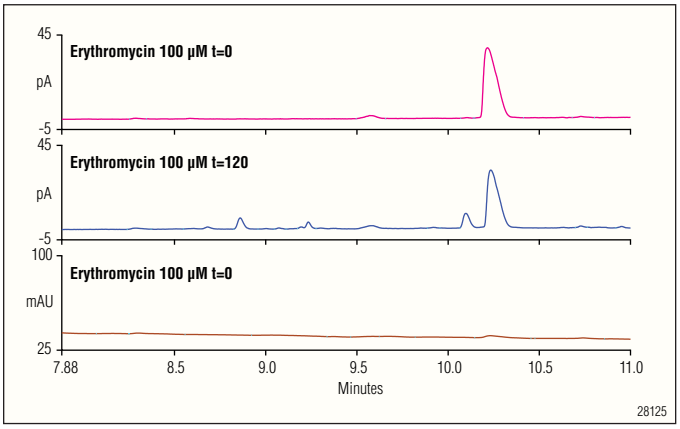

Figure 7. Results for CAD pre- and postincubation and UV at 210 nm pre-incubation of the 100 µM erythromycin sample.

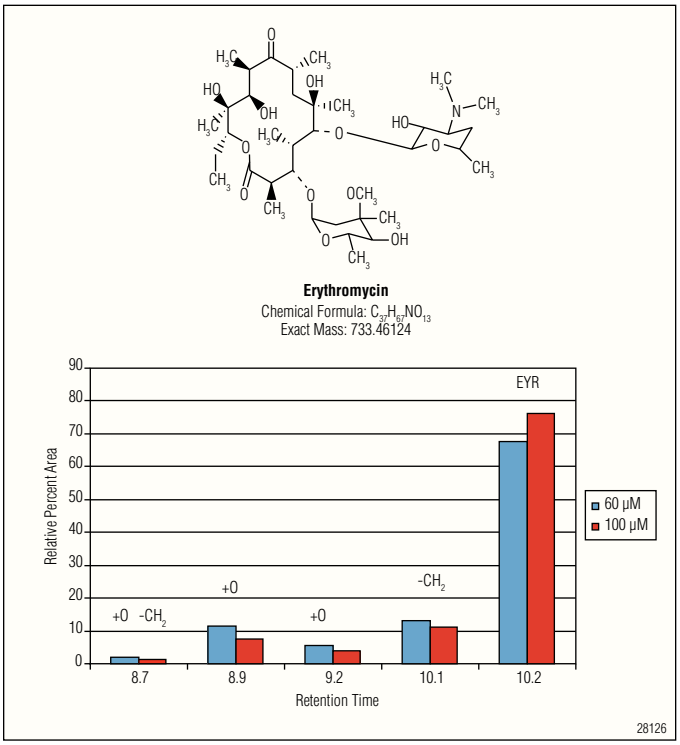

Figure 8. Structure (top) of erythromycin. Plot of relative area response of the four metabolites and the parent peak with CAD detection (bottom).

DISCUSSION

The results discussed here where obtained in collaboration with BMS. The optimization of the system and development of the methods were ongoing throughout the work. Several components were identified as crucial to maintaining quality data on both the CAD and MS. The column diameter and load volume was important as some of this work was done on a system with a 400 bar HPLC. The setup of the flow splitting when running the LC-CAD-MS system was determine to be the most crucial factor. The flow splitter must be positioned close to the MS inlet with a 0.004 inch or smaller i.d. tubing from the splitter to the MS (Figure 2). The optimal flow-split range is estimated being between 100 to 200 µL/min flowing to the MS and the remaining flow to the CAD. Because of the levels of sensitivity required for these analyses, a minimum flow rate of 500 µL/min is recommend for the CAD.

The first example, buspirone, was chosen because it is a well characterized example, has a strong UV chromophore, and the standard material is readily available. The post HLM incubation of buspirone for both the CAD and UV (Figure 4) show a similar metabolite profile. The five major metabolites shown in Figure 3 were quantified as relative peak areas from the initial 60 µM buspirone sample. A sixth, minor metabolite was a volatile fragment of the buspirone and was not detected on the CAD. The standards for the buspirone and the five metabolites were prepared and analyzed at 15 µM concentration. The results for the two UV wavelengths, the CAD, and the MS are shown in Figure 5. The greatest variability was found in the UV trace at 254 nm with an RSD of 53% followed by the MS at 37%. The CAD and low wavelength UV had similar deviations of 20 and 17%, respectively.

The CAD, although not a linear detector over the full dynamic range, is typically linear from its limit of quantification to ~500 ng on column. In Figure 6 the buspirone standard was analyzed using a UHPLC method from 3.9 to 124 ng on column. The six-point calibration curve exhibited excellent linearity over this range with a correlation coefficient of 0.9994. The concentration range of interest needed for the testing of drug metabolites typically falls in this mass-on-column range, which is linear for the CAD independent of analyte.

The second example, erythomycin, was chosen because it is representative of an array of drug candidates that fall into the nonvolatile analyte category with weak or no UV chromophores. As shown in Figure 7, the pre-incubation 100 µM standard has virtually no response with low wavelength UV. After incubation, the CAD was able to detect the four major metabolites observed by MS. The relative area response for those four metabolites and the parent peak were evaluated at the 100 and 60 µM levels (Figure 8). As expected, the relative intensities of the metabolites increased at the lower concentration over the same incubation time.

The level of detection on the CAD for the buspirone was determined to be ~1 ng on column with the short run UHPLC chemistry. This is typical for nonvolatile analytes under optimized conditions. Depending on molecular weight of the compound and the injection volume used, the molar concentration limit of detection can vary. While more sensitive than other nebulizer-based detection techniques, charged aerosol detection still may not provide sufficient sensitivity to meet the detection levels needed for in vivo studies. The "MetPro" practice, advocated by J. Joseph (2009), of normalizing the MS metabolite ratios at a single, higher-level point with UV data and then transferring that to the MS results at the low levels has been employed in previous work.[4] This was examined with the CAD results during the calibration and is further described in Cai, et al. (2010).[2] This offers a method to reduce the analytical deviations due to ionization variability while keeping the sensitivity offered by the mass specrometer.

CONCLUSION

The UHPLC-UV-CAD-MS system described in this study was able to provide crucial information needed to meet requirements of the new FDA MIST initiative. The use of charged aerosol detection as confirmation of low wavelength UV quantification for chromophoric compounds can provide additional confidence to the results. In areas where more specific wavelengths are used, such as the buspirone 254 nm, the CAD can highlight large under- or overestimation such as shown with metabolite 5. The area where this technology is most applicable is for parent or drug compounds that do not contain a strong UV chromophore. In these cases, laboratories are often left with no other option but to accept the MS quantification during the early drug discovery stages. This could lead to major issues in later drug development stages. The CAD, while not a stand-alone solution, can provide those additional pieces of data to make accurate interpretations of in vitro data without excessive cost or time requirements.

ACKNOWLEDGMENTS

We would like to thank Hong Cai and colleagues Bristoyl-Myers Squibb for their work on this collaboration. Generation of the data used would not have been possible without their help.

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