Increasing Quality While Maintaining Efficiency in Drug Chemistry with DART-TOF MS Screening

Application Note

Forensics, Drug Chemistry

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Abstract

A sample prescreening method for forensic analyses was developed using DART-TOF MS with continuous calibration. This method provides reduced workflow and minimal cycle times, while improving screening accuracy for better confirmation tests. The method eliminates sample preparation and subjective screening methods for some classes of drugs. As a result, using this screening method, drug chemistry labs can improve productivity and confidence in analytical results.

Agilent Technologies

Introduction

Forensic science and its applications in the criminal justice system have evolved through the years. Regulations for forensic lab work have become increasingly strict and some, such as those described in ISO 17025 International Standards, require immediate implementation. These include sampling plans, documented reviewable data, and method validation. The well-defined standards are driving organizations such as the National Academy of Sciences (NAS) to develop recommendations on how forensic labs can meet them. Some of the recommendations include minimizing human bias in measurement methods, and setting analytical standards for forensic practices, especially case processing.

In the past, traditional sample analysis workflow began with a visual examination, weight measurement, as well as presumptive tests including color, microcrystal, TLC, GC, and LC as prescreening (Figure 1). Visual and color tests are subjective in nature, and do not have measurable controls, which is a stipulation in the NAS and ISO regulations. In addition, these tests do not provide reviewable data. Extra GC/MS screens were performed to determine the target analytes, which increased analysis time.

In response to the NAS and ISO 17025, the Alabama Department of Forensic Science (ADFS) has rewritten operating procedures to require reviewable data at all phases of the forensic analysis. A method was developed using an Agilent 6224 TOF LC/MS, with a DART ion source for preliminary screening of drug samples prior to analysis. This method has been proven to provide reliable and accurate results, while changing the workflow to minimize cycle time and necessary manpower, increasing lab output.

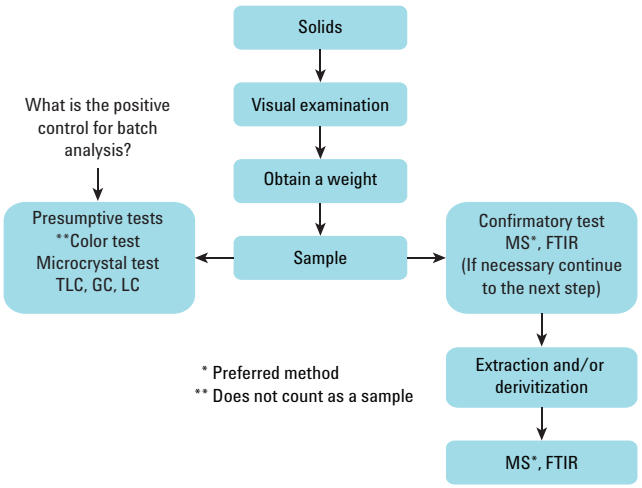

Figure 1. Typical flow chart for case analysis.

Experimental

The objective of this study was to develop a method capable of high mass accuracy with calibration but without extraction, which could maximize throughput. The studies were performed using actual case work from a local lab. The method was developed using an Agilent 6224 TOF LC/MS coupled with a DART ion source. PEEK tubing (0.005 in id, red) and stainless steel tubing (0.010 in id) (Figure 3), along with a syringe pump (WPI SP100I) were attached to the TOF to provide continuous flow of calibration solution. This was tested to verify that the method can provide exact mass measurements for putative compound identification.

Figure 2 shows a graphical interpretation of DART ionization, which is used prior to the TOF LC/MS analysis. As the figure illustrates, in positive ion mode, metastable helium ionizes atmospheric water, which then donates a proton to the sample. Figure 3 shows the next step, in which reference ions are added continuously with each sample using the syringe pump and PEEK tubing. This arrangement allows real-time analysis as well as a control in every data file, since calibration is performed continuously as the samples are analyzed.

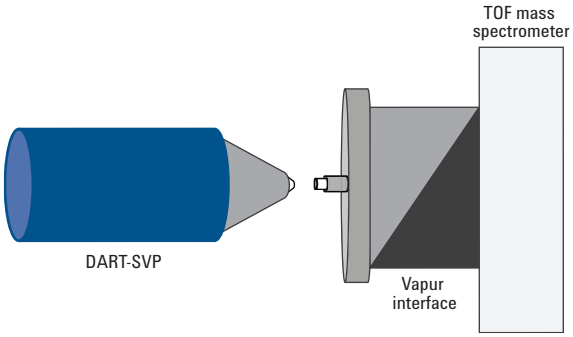

Figure 2. System overview – DART ionization.

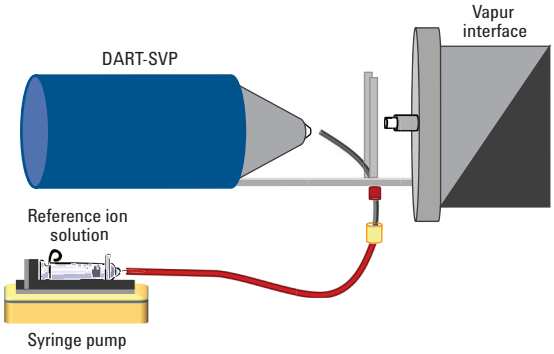

Figure 3. System overview – reference solution introduction.

Instrument conditions for the Agilent 6224 TOF LC/MS are listed in Table 1.

Table 1. Instrument Conditions

Time segment 1	
Acquisition mode MS1	
Min range (*m/z*)	60
Max range (*m/z*)	600
Scan rate (spectra/sec)	1.00
Source parameters	
Gas temperature	325 °C
Gas flow	10 L/min
Nebulizer	0 psig
Scan segments	
Segment 1	Positive ion polarity
Scan segment 1	
Scan source parameters	
VCap	1,000
Fragmentor	175
Skimmer 1	65
Octopole RF peak	250
DART parameter settings	
Positive mode	
Gas heater temperature	350 °C
DART distance	3.0 cm

Sample preparation

This method does not require sample preparation, and raw samples are preferred. The method was tested using a variety of samples such as soda, tablets, contaminated candy, currency, bottles, and powders. The only requirement for the method is that the sample fit in the sampling area of the DART-TOF system.

Results and Discussion

This method of prescreening provided mass accuracy within 5 mDa of the theoretical mass of the target compounds when calibration was performed with analysis. Raw samples provided the most accurate results, eliminating the need for extraction. The throughput was high since typical analysis time for a sample is 2 minutes, while the total analysis time is 4 minutes, including analysis of a sample blank.

The benefits of this method are quantifiable. The DART-TOF provides reviewable data for case screenings. During the course of the study, it was determined that some samples showed negative results when screened with traditional techniques, yet screened positive with DART-TOF MS. In addition, compounds that do not have traditional screening techniques available are easily analyzed with this method. Varying results depend upon sampling method, solvent choice, and LOD. Since reference compounds are introduced to the DART-TOF in a continuous aqueous flow, this is considered "positive control" in every run, providing for a control in every data file.

Figure 4 shows an example of the results from one run, reference ions appear in the entire spectra.

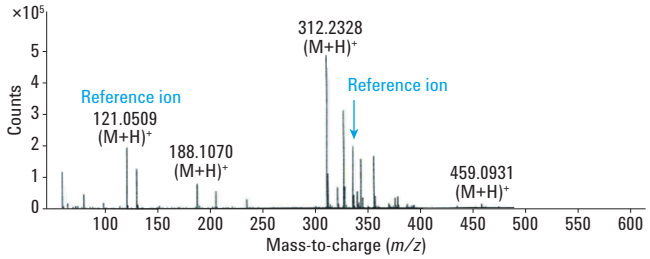

Figure 4. DART-TOF MS data – reference ions.

Compare the data shown in Table 2 with the spectra shown in Figure 5. As you can see, many compounds have the same molecular weight. This is why the DART-TOF is a screen-only method, as opposed to one used for analysis. The DART-TOF provides an accurate reference mass range to be used for the confirmation GC/MS analysis. This limitation of the TOF could be overcome through the use a Q-TOF instrument that provides MS/MS spectral information along with accurate mass data.

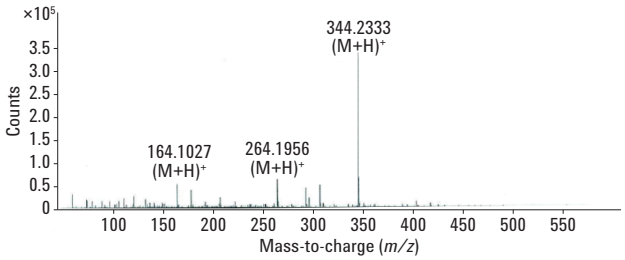

Figure 5. DART-TOF MS spectra.

Compound name	Reference molecular formula	Reference mass	Mass	Difference
Purine	$C_5H_4N_4$	120.0436	120.0438	–0.21
Methcathinone	$C_{10}H_{13}NO$	163.0997	163.0954	4.29
Methcathinone	$C_{10}H_{13}NO$	163.0997	163.0954	4.29
6-APDB-(6-(2-aminopropyl)-2,3-dihydrobenzofuran)	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
4-MMC(Mephedrone)(4-methylmethcathinone)	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
3-Methylmethcathinone (+2 isomers)	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
Buphedrone	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
Phenmetrazine	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
MMAI(5-methoxy-6-methyl-2-aminoindane)	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
Buphedrone (alpha-ethylamino-propiophenone)	$C_{11}H_{15}NO$	177.1154	177.1109	4.48
Tramadol	$C_{16}H_{25}NO_2$	263.1885	263.1876	0.89
Dibucaine	$C_{20}H_{29}N_3O_2$	343.226	343.226	–0.05
Nupercaine	$C_{20}H_{29}N_3O_2$	343.226	343.226	–0.05

Table 2. DART-TOF MS Results – Peak List

The DART-TOF method was tested on a variety of substances and sample types. This technique is considered an acceptable method for prescreening, since the test results met the most important qualifications. All matches had an abundance greater than 5%, and all mass matches were within ± 5 mDa. Reference ions are present in blanks, negative controls, and samples. They also meet the abundance and mass criteria. Sample blanks and negative controls are free of any controlled substances and previous sample carryover, and also contain reference ions. Finally, all resulting compound peaks were at least three times the baseline.

As a result of the new prescreening method qualification, a workflow was developed for drug analysis case work. Figure 6 depicts a flow chart of the workflow. The diagram shows that cases are now triaged by a prescreen team prior to analysis instead of each case screened separately by the analyst as in traditional case analyses. DART-TOF is the prescreening technique used for any sample containing powders, liquids, or unknown plant materials. After triage, a separate analyst handles the actual drug screening. This new workflow limits the tasks necessary for each analyst, allowing the screen team to sort cases into batches for the analyst prior to the run. This, in turn, adds redundancy to the analysis, and reduces employee workload.

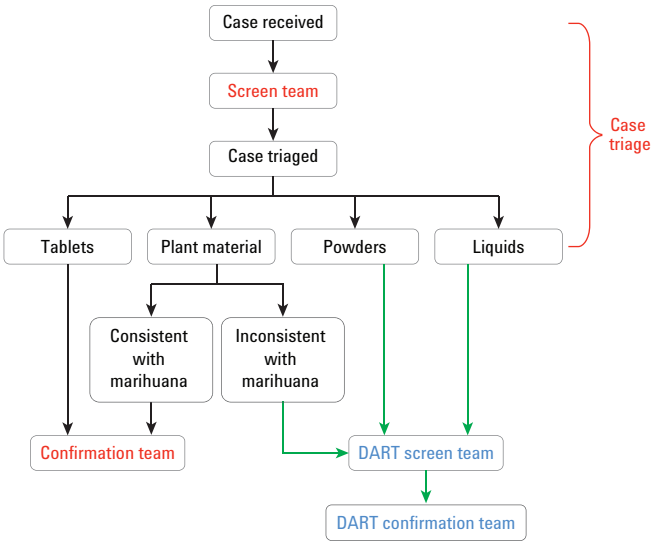

Figure 6. New workflow.

Table 3 shows the improvement in productivity achieved by the test lab, once this method was implemented. As the data indicates, the number of cases processed per analyst increased dramatically from 2012-2013 with the introduction of triage with DART-TOF.

Table 3. Triage Workflow Shows Increase in Average Numbers per Analyst

Month	Analyst no.	Approved	Avg/analyst	Items
June 13	7	630	90	987.25
July 13	6	507	85	953
June 12	7	370	53	1076
July 12	6	439	73	1514

As demonstrated by the test lab, the new workflow allows one analyst to prescreen approximately 40 items in 5 hours, and perform data analysis simultaneously. Table 4 shows the increase in approved cases from 2012–2013.

Table 4. DART Screen Workflow Improvement

Month	Analyst no.	Approved	Items
June 13	1	200	172
July 13	1	165	107.5
June 12	1	26	171
July 12	1	106	160

Month	Analyst no.	Reported
October 13	10	800
November 13	10	800
October 12	7	476
November 12	7	397

This process is a relatively new workflow for drug chemistry labs, and has been implemented in the test lab only. Since there is a limited number of trained analysts at this site, this translates to a limited number of scientists to report and review the data. The transition from a single analyst to a team of analysts per case has improved productivity. Cases can be effectively sorted into batches following the first sampling without extraction, allowing the confirmation analyst to test an entire batch of cases in sequence. This test generates reviewable data that can be recorded and stored with the batch for further reference. Since a minimum of two scientists are involved in each case, there is increased confidence in analyte determination as an additional scientist now confirms the evidence description.

Conclusion

This application note shows that the DART-TOF MS prescreening method provides advantages over traditional screening techniques in forensic drug chemistry labs. The method provides a single prescreening test for a wide range of analytes, as well as reviewable data that can be recorded and stored with the case records. It also provides the ability to prescreen for emerging analytes that do not have a traditional screening technique, such as synthetic cannabinoids. Use of this prescreening minimizes cycle time, by introducing continuous calibration with every run. The workflow developed as a result of this method improves productivity and confidence in analytical results.

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