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# THE APPLICATION OF WATERS ATMOSPHERIC PRESSURE SOLIDS ANALYSIS PROBE (ASAP) TO THE ANALYSIS OF PHARMACEUTICAL FORMULATIONS AND METABOLITES IN URINE

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## INTRODUCTION

The last few years have seen the introduction of several new techniques for ionization of samples under ambient conditions. The most commonly cited of these being Desorption Electrospray Ionization (DESI)[1] , invented by Professor R. Graham Cooks, *et al.* at Purdue University and Direct Analysis in Real Time (DART)[2] developed by Cody, *et al.* The major benefit of these ambient desorption ionization technologies is that they require little or no sample preparation.

A low cost alternative, the Atmospheric Pressure Solids Analysis Probe (ASAP)[3], introduced by McEwen *et al.*, has also been shown to be a useful tool for the rapid direct analysis of volatile and semi-volatile solid and liquid samples using atmospheric pressure ionization. The ASAP technique depends on heated nitrogen desolvation gas to vaporize the sample and a corona discharge for ionization. It is capable of ionizing low polarity compounds not amenable to ESI, APCI, and APPI at high sensitivity and can also be used for the analysis of complex samples without the need for any sample preparation.

Here we introduce the Waters® Atmospheric Pressure Solids Analysis Probe*. ASAP is readily fitted to an atmospheric pressure ionization (API) source by the simple replacement of either the ESI or APCI probe and the fitting of a corona discharge pin. This simplifies the technique when compared to either DESI or DART for the analysis of low molecular weight compounds. The proximity of the sample to the point of ionization and the MS inlet improves sensitivity while the source housing is enclosed for safety.

In this technical note, the technique has been applied to direct analysis of pharmaceutical tablet formulations, metabolites in urine, and a Gelucire wax, which is used as a bioavailability enhancer.

**Waters Atmospheric Pressure Solids Analysis Probe (ASAP), has been developed under license to M&M Mass Spec Consulting LLC, Hockessin, Delaware, USA (patent pending).*

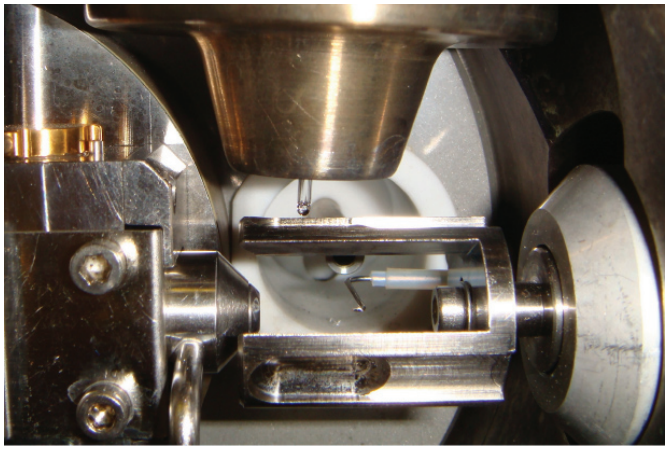

*Figure 1. ASAP fitted to the LCT Premier XE System.*

## EXPERIMENTAL

### Sample preparation

Samples were introduced on a sealed glass melting point capillary and vaporized in a stream of heated nitrogen. The temperature of the nitrogen was ramped to control the vaporization of components in the complex mixtures. The sample in the gas phase was ionized by proximity to a corona discharge needle. Ions then passed from the atmospheric pressure region into the mass spectrometer.

Pharmaceutical tablets were cut with a scalpel to expose a clean surface with no tablet coating. A sample capillary was then wiped across this surface and analyzed directly.

1 µL of a urine sample taken from a patient four hours after dosing with paracetamol (1000 mg) and dihydrocodeine (30 mg) was loaded directly onto a glass capillary without any sample pre-treatment.

The Gelucire wax was smeared on a capillary and the excess removed with a tissue.

### MS conditions

The samples were analyzed on an LCT Premier™ XE System operated in ESCi® mode, which allowed control of both the desolvation gas heater and the corona discharge current. This configuration also permitted the use of LockSpray™ for exact mass measurements as shown in Figure 1.

| | |
|---|---|
| MS System: | Waters LCT Premier XE System |
| Ionization Mode: | ESCi |
| Sample Cone: | 30 V |
| Source Temperature: | 120 °C |
| Desolvation Temperature: | 50 to 450 °C |
| Desolvation Gas: | 500 L/hr |
| Capillary Voltage (ESI): | 3000 V |
| Corona Current (APCI): | 5 µA |
| Mass Range: | m/z 50 to 1000 |
| Lock Reference (ESI): | leucine enkephalin |
| | 200 pg/µL at 3 µL/min |
| Exact Mass: | 556.2771 |

## RESULTS AND DISCUSSION

### Analysis of tablet formulations

Figure 2 shows ASAP results obtained from direct analysis of a Zantac (ranitidine) 75 mg tablet used in the treatment of heartburn and acid indigestion. The results from the analysis of a 10 mg temazepam tablet, an anti-anxiety drug, are shown in Figure 3.

In addition to the active pharmaceutical ingredients, both tablets contained a range of excipients including microcrystalline cellulose, magnesium stearate, hypromellose, and titanium dioxide. Analysis of complex tablet formulations such as these by LC/MS and GC/MS would require a lengthy sample preparation procedure prior to analysis. ASAP was able to provide clean spectra of the active pharmaceutical ingredient along with exact mass data and elemental compositions without any sample preparation or chromatographic separation, even when only present at a few milligrams in the formulated tablet.

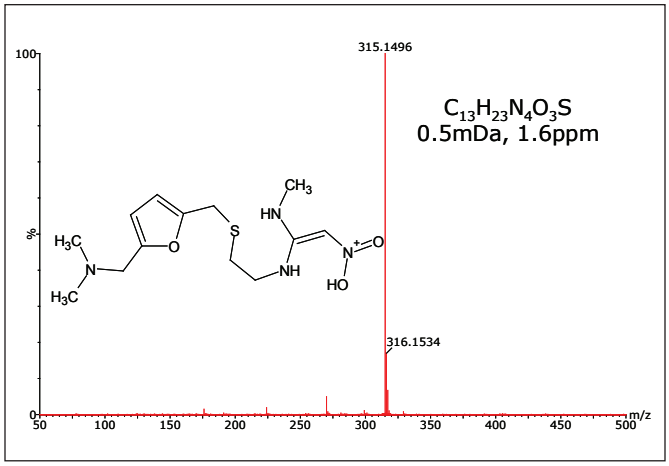


*Figure 2. Direct analysis of Zantac (ranitidine) 75 mg tablet used in the treatment of acid indigestion.*

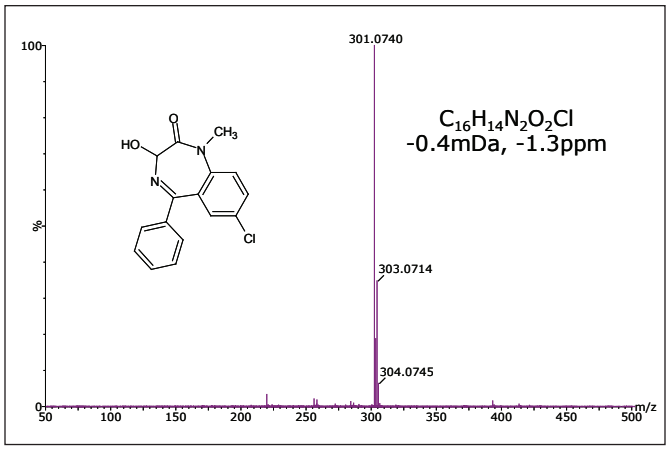


*Figure 3. Direct analysis of temazepam 10 mg tablet used in the treatment of anxiety.*

This demonstrates the potential use of ASAP for the rapid analysis of tablets to combat the problem of counterfeit drugs, which might contain no active pharmaceutical ingredient, or for forensic applications. It is also a useful alternative to an EI/CI solids probe for confirmation of molecular weight of synthesized products.

## Analysis of drugs and metabolites in urine

In this example, a patient was administered paracetamol (1000 mg) and dihydrocodeine (30 mg). A urine sample was collected four hours post-dose.

The elemental composition report for the direct analysis of a dihydrocodeine (30 mg) tablet is shown in Figure 4. As was observed with the previous examples, the most significant peak in the spectrum was from the active pharmaceutical ingredient. At the temperature employed for the analysis, a peak corresponding to the loss of water was also observed as confirmed by exact mass measurements (< 5 ppm) and the elemental composition report.

A 1 µL aliquot of neat urine from four hours post-dose was pipetted onto the ASAP melting point tube and introduced into the source. The temperature was then ramped from 50 to 350 °C. As the temperature increased, different components of the urine were vaporized. At temperatures > 200 °C, paracetamol and the endogenous metabolite creatinine were the major species observed, as shown in Figure 5a. The dihydrocodeine was also apparent and closer examination of the averaged spectrum, see Figure 5b, also showed the presence of its metabolite dihydromorphine, which resulted from demethylation.

A spectrum obtained at a temperature of < 150 °C showed the presence of caffeine and the beginning of the paracetamol vaporization profile, as shown in Figure 6. Again, the identification was confirmed by exact mass measurement.

These results showed that the technique does not undergo suppression effects associated with electrospray and APCI, and that valuable information on the drugs taken and metabolites formed could be obtained from 1 µL of neat urine. The sensitivity of this technique was apparent when it was considered that the metabolite of a drug dosed at 30 mg could be detected in 1 µL of urine.

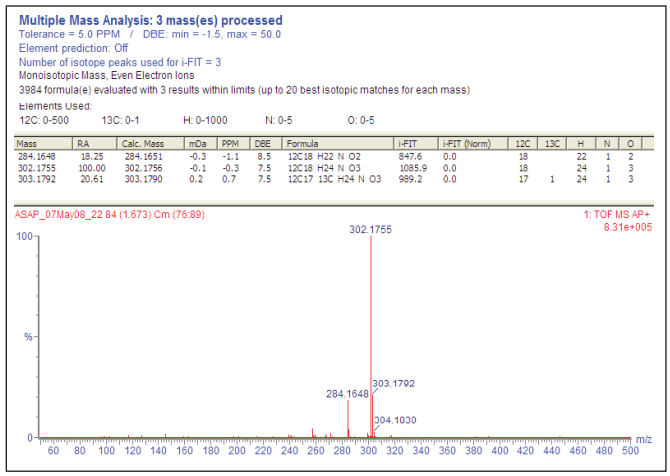


*Figure 4. ASAP of dihydrocodeine 30 mg tablet.*

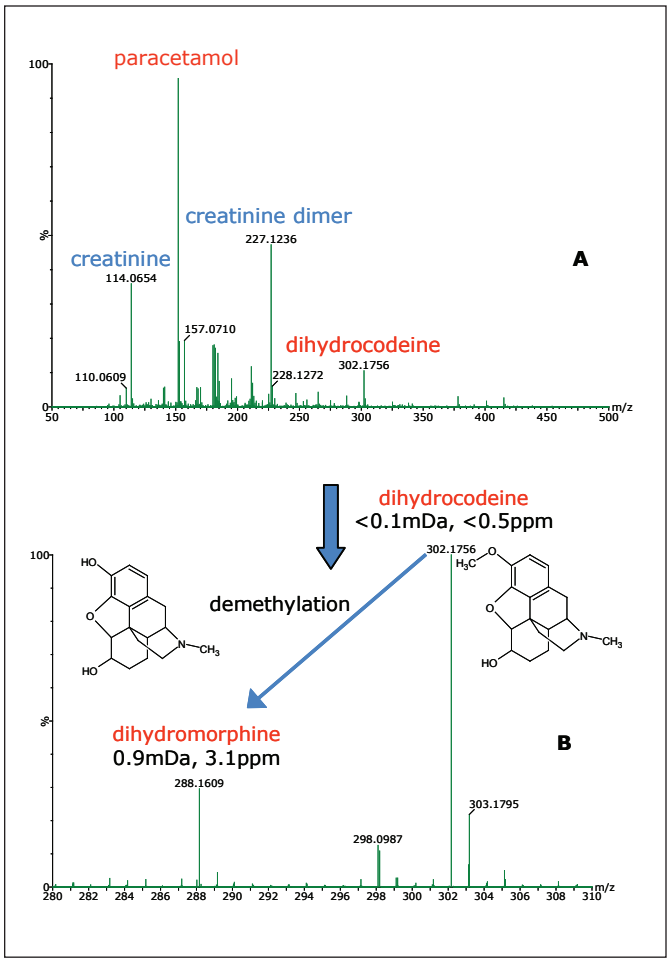


*Figure 5. a) ASAP of neat urine sample. b) Expanded region (m/z 280-310).*

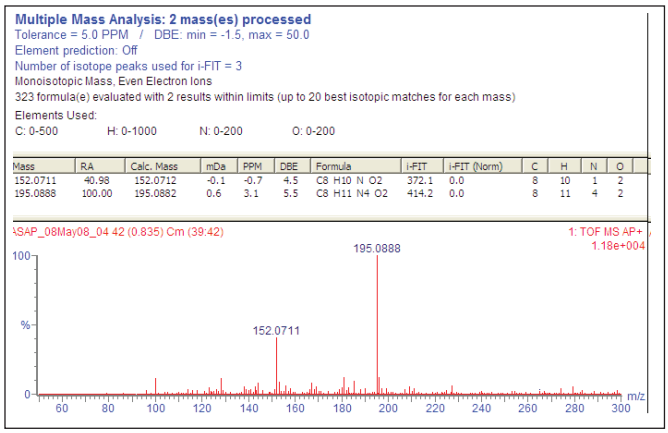


*Figure 6. ASAP analysis of neat urine sample. Spectrum obtained from the average of four scans at < 150 °C.*

## Analysis of polymeric wax

Gelucire is a semi-solid excipient frequently used in the pharmaceutical industry. It is a mixture of polyethylene glycol (PEG) and glyceryl esters of long chain fatty acids and is commonly used in capsule formulations to enhance drug solubility. The Gelucire wax spectrum in Figure 7 shows a distinct series of peaks 44 Da apart associated with PEGs and another 28 Da apart corresponding to $(C_2H_4)_n$.

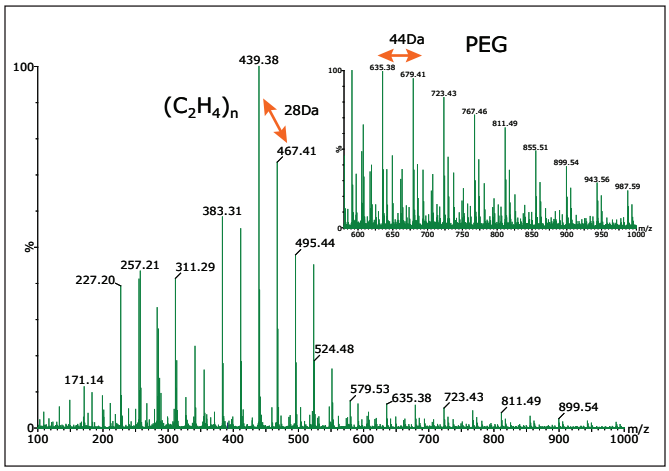


*Figure 7. ASAP of Gelucire wax with the region m/z 580-1000 inset showing PEG peaks 44 Da apart.*

## CONCLUSION

Complex samples were analyzed without any sample pre-treatment or chromatographic separation. ASAP was successfully used to detect the active pharmaceutical ingredients in complex tablet formulations and xenobiotics in neat urine, as well as to characterize a polymer wax used as a bioavailabilty enhancer in capsule formulations.

ASAP analyses offers good sensitivity, as evidenced by its ability to detect low level drug metabolites in the urine sample due to the proximity of the sample capillary to the point of ionization and the mass spectrometer inlet.

The technique is applicable to the analysis of both solid and liquid samples, as long as they have sufficient volatility. It is particularly suitable for the analysis of low polarity or non-polar compounds. This is a good alternative to an EI/CI solids probe with the advantage of no vacuum lock. The enclosed source also ensures the users safety when samples are volatilized.

The coupling of ASAP with a Time-of-Flight (ToF) analyzer made the identification of unknowns possible through exact mass measurements and elemental composition determination with iFIT™ for isotope ratio comparison. This combination has been shown to be ideal for the rapid confirmation of the molecular weights of a range of compounds in complex samples typical of those encountered in the pharmaceutical industry.

## References

1. Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Science, 2004, 306, 471.

2. R.B. Cody, J.A. Laramee, H.D. Durst, Anal. Chem. 2005, 77, 2297.

3. C.N. McEwen, R.G. McKay, B.S. Larsen, Anal. Chem. 2005, 77, 7826.



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