Qualitative Detection of Drugs in Sweat Using Thermal Extraction Ion Source

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Introduction

We were recently given access to two drugs used to treat chronic lymphocytic leukaemia (CLL). We were curious to see whether these drugs were amenable to direct analysis without chromatography, utilising the Sciex API 3200 triple quadrupole mass spectrometer coupled to the Mass Spec Analytical (MSA) TEIS Ambient ionisation source. The TEIS (Thermal Extraction Ion Source) is an APCI source based on thermal desorption from two heated plates. An early version was described by Ebejer[1]. Sample introduction can be effected without chromatography, either by means of paper swabs of solid material, toothpicks or in solution via syringe[2].

Further, if a method could be developed, to investigate whether the compounds could be detected in an individuals sweat. Sweat has been advocated as a suitable matrix for the detection of traces of drugs of abuse and their metabolites[3].

Ibrutinib (Imbruvica) is a small molecule drug ($C_{25}H_{24}N_6O_2$) that binds permanently to a protein, Bruton's tyrosine kinase (BTK), that is important in B cells; the drug is used to treat B cell cancers like mantle cell lymphoma, chronic lymphocytic leukaemia (CLL), and Waldenström's macroglobulinemia, a form of non-Hodgkin's lymphoma. The structure, with monoisotopic mass 440.196075 Da is shown in Figure 1 below:

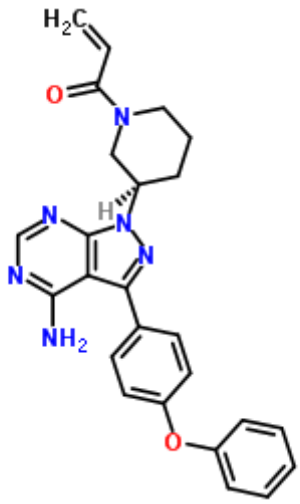


Figure 1: Ibrutinib structure

Allopurinol, sold under the brand name Zyloprim amongst others, is a medication used to decrease high blood uric acid levels. It is specifically used to prevent gout, prevent specific types of kidney stones, and for the high uric acid levels that can occur with chemotherapy. Its molecular formula is $C_5H_4N_4O$, its monoisotopic mass is 136.038513 Da, and its structure is shown in Figure 2 below:

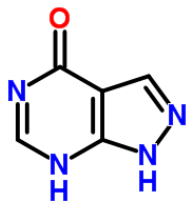


Figure 2: Allopurinol structure

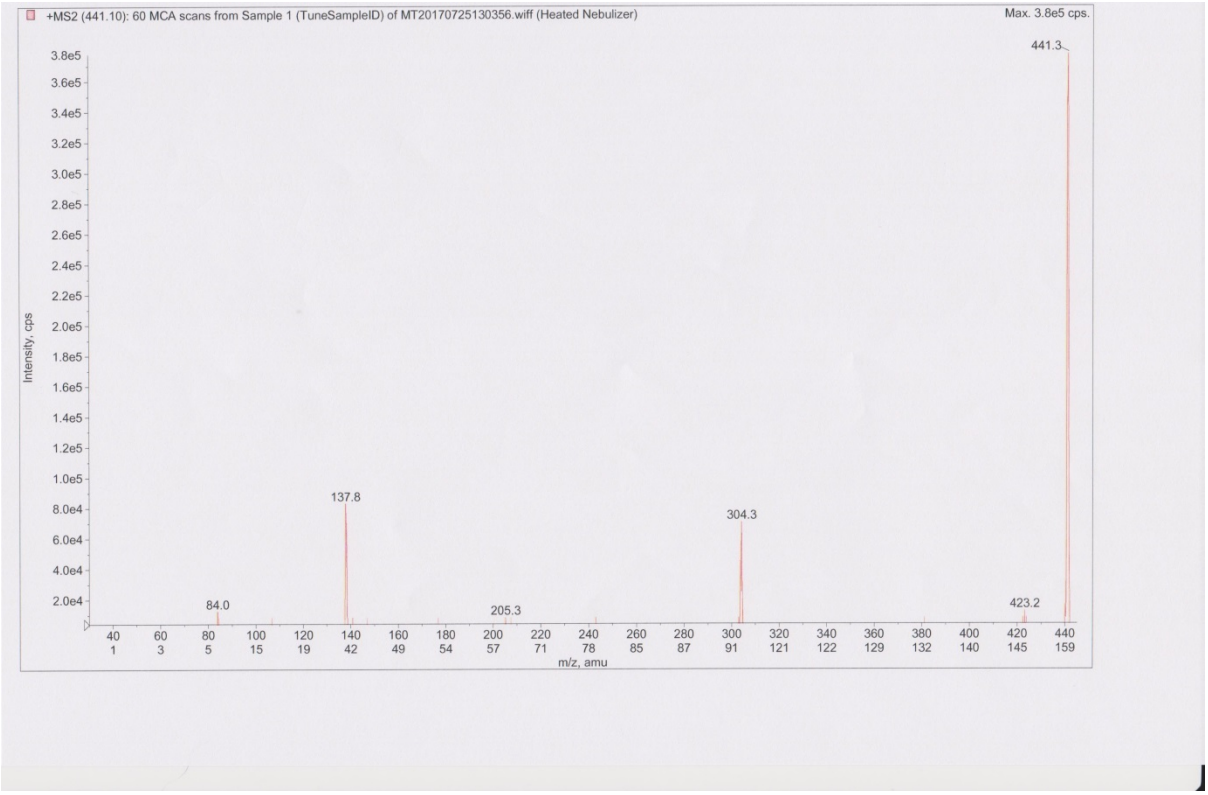
Methodology

There being no certified reference materials available as standards, we resorted to using the prescribed drugs themselves as an alternative.

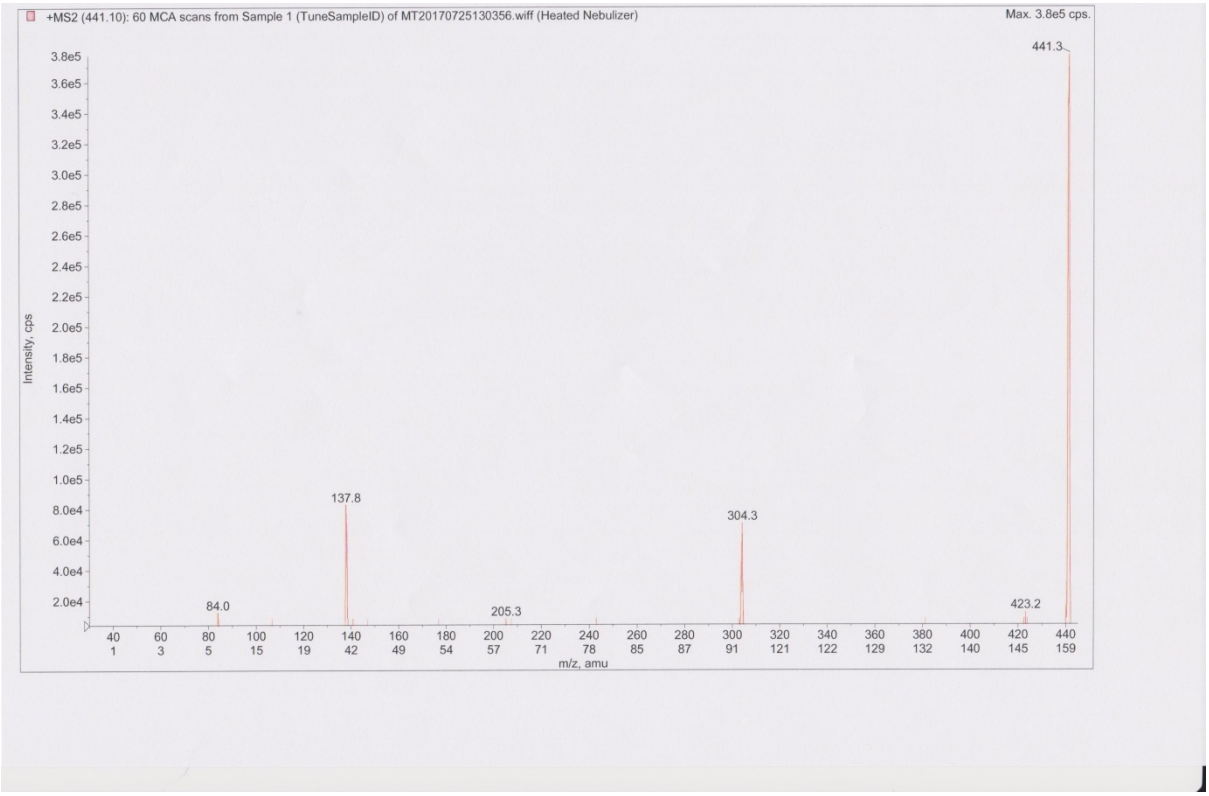
Ibrutinib

Ibrutinib is extremely expensive, and we could not justify opening a capsule and 'wasting' the contents. The plastic container in which they are supplied holds 12.6 g of the drug (90 capsules) at the start of the month (albeit isolated from the plastic container by the capsule). We rationalised that there might be sufficient material adhering to the plastic to use as a 'standard'. We therefore swabbed the inner surface of the empty container at the end of the month, using a disposable cotton swab wetted with methanol. We dipped the swab into a vial containing approximately 1 mL of methanol, and found that this contained sufficient of the material (presumed ibrutinib) for our investigations.

We chose not to investigate whether the mass spectrum of ibrutinib had been reported in the literature, in order not to be prejudiced by the approaches used. We arbitrarily set the temperature of the TEIS-3200 heated inlet at 285°C, a temperature which has been found to work well for a wide range of compounds. Guessing from the structure that ibrutinib would most likely form an $[M+H]^+$ protonated molecule, We started with a Q1 scan between *m/z* 435 and *m/z* 445, with only ambient air being drawn into the source for 1 min. We then repeated this 1 minute scan whilst repeatedly injecting the unquantified presumed ibrutinib solution (7 x 5 µL via Hamilton syringe). These injections were performed manually; slowly such that any fluctuations in the rate of sample introduction would be averaged out by the scanning speed of the mass spectrometer. The peak at *m/z* 441, corresponding to the $[M+H]^+$ protonated molecule of presumed ibrutinib, was seen to be augmented by more than an order of magnitude, suggesting that the protonated molecule of ibrutinib was indeed present. We then acquired a product ion spectrum from m/z 441 for 1 min, first allowing only ambient air into the plenum chamber, and observed that nothing significant was detected, before repeating the product ion scan acquisition repeatedly injecting the unquantified presumed ibrutinib solution (7 x 5 µL via Hamilton syringe) as before. The Collision Energy selected for these experiments was again arbitrarily set at 30 eV. The resultant product ion spectrum for presumed ibrutinib is shown



below:



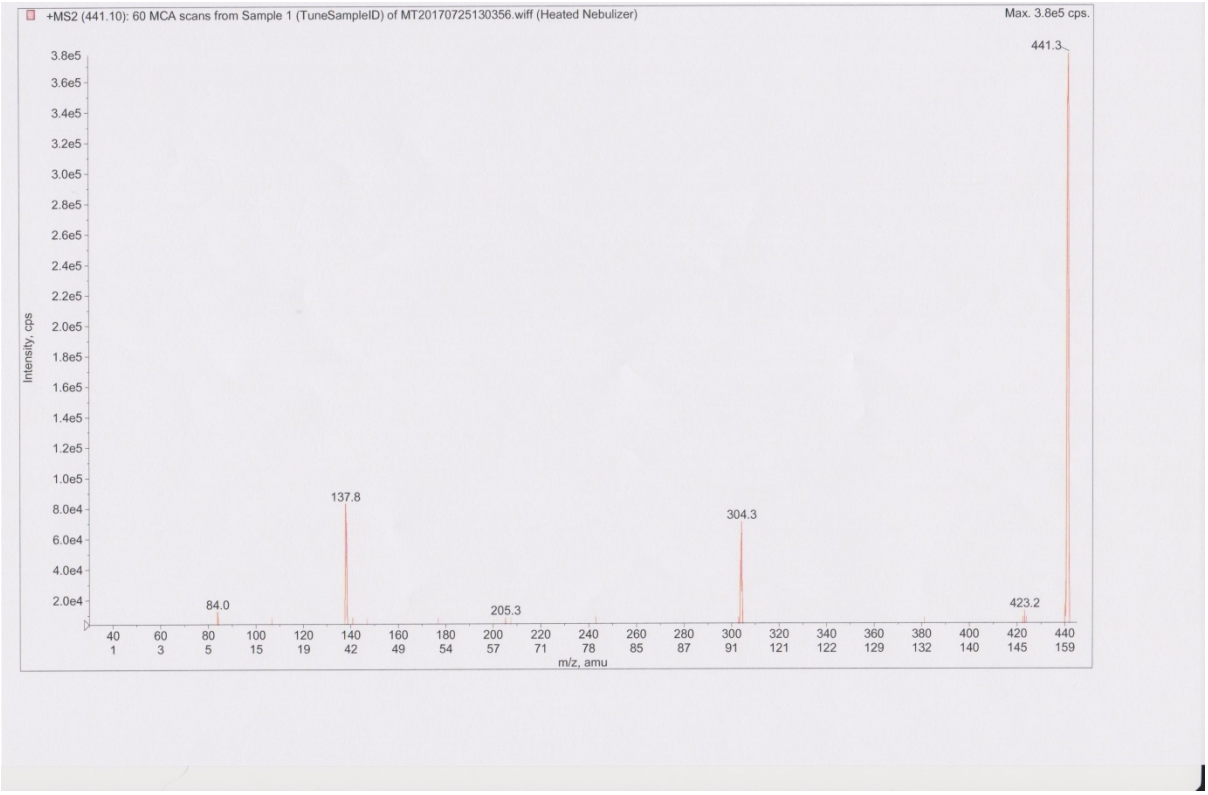


Figure 3: Ibrutinib Product Ion Spectrum

It can be seen that there are two major fragments to *m/z* 304 and *m/z* 138, both most likely accounted for by fragmentation of the bond between the pyrazole ring and the piperidine ring, with charge retention on the two different fragments. The minor fragment at *m/z* 84 can be postulated to be further fragmentation of the 2-propen-1-one group. Only after acquiring this spectrum did we compare with literature reports for ibrutinib, and identical fragmentations have been reported[4].

The next step was to establish an MRM method, utilising the transitions from *m/z* 441 to *m/z* 304 and *m/z* 138. A piece of normal printer paper was cut into pieces measuring approximately 10 cm x 6 cm. Figure 4 depicts an MRM analytical run of approximately 1.3 minutes. During this time two paper swabs were blanked by being inserted into the TEIS thermal desorber (at approx. 0.15 mins. and 0.4 mins.), followed by swabs from the side of the nose (0.6 mins.), and the forehead (0.85 mins.) of the individual who is prescribed the medication. A clear response to the presumed ibrutinib is observed from both skin swabs.

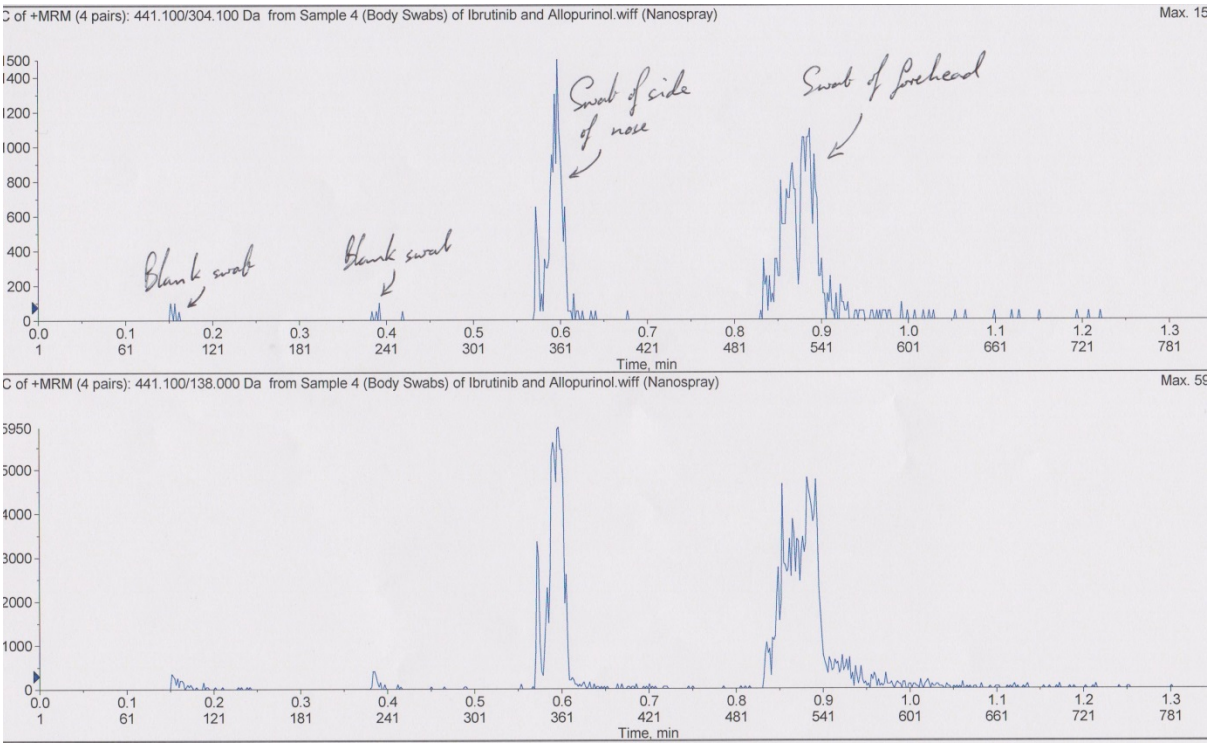


Figure 4: MRM of Ibrutinib

Allopurinol

Allopurinol is supplied in 'blister packs' containing tablets of 300 mg. We rationalised that there might be sufficient material adhering to the plastic to use as a 'standard'. We therefore swabbed the inner surface of two of the empty cavities, using a disposable cotton swab wetted with methanol. We dipped the swab into a vial containing approximately 1 mL of methanol, and found that this contained sufficient of the material (presumed allopurinol) for our investigations.

We again arbitrarily set the temperature of the TEIS-3200 heated inlet at 285°C. Guessing from the structure that allopurinol would most likely form an $[M+H]^+$ protonated molecule, we started with a Q1 scan between *m/z* 130 and *m/z* 140, with only ambient air being drawn into the source for 1 min. We then repeated this 1 minute scan whilst repeatedly injecting the unquantified presumed allopurinol solution (9 x 5 µL via Hamilton syringe). The peak at *m/z* 137, corresponding to the $[M+H]^+$ protonated molecule of presumed allopurinol, was seen to be augmented by more than an order of magnitude, suggesting that the protonated molecule of allopurinol was indeed present. We then acquired a product ion spectrum from m/z 137 for 1 min, first allowing only ambient air into the plenum chamber, and observed that nothing significant was detected, before repeating the product ion scan acquisition repeatedly injecting the unquantified presumed allopurinol solution (7 x 5 µL via Hamilton syringe) as before. The Collision Energy selected for these experiments was again arbitrarily set at 30 eV. The resultant product ion spectrum for presumed allopurinol is shown in Figure 5 below:

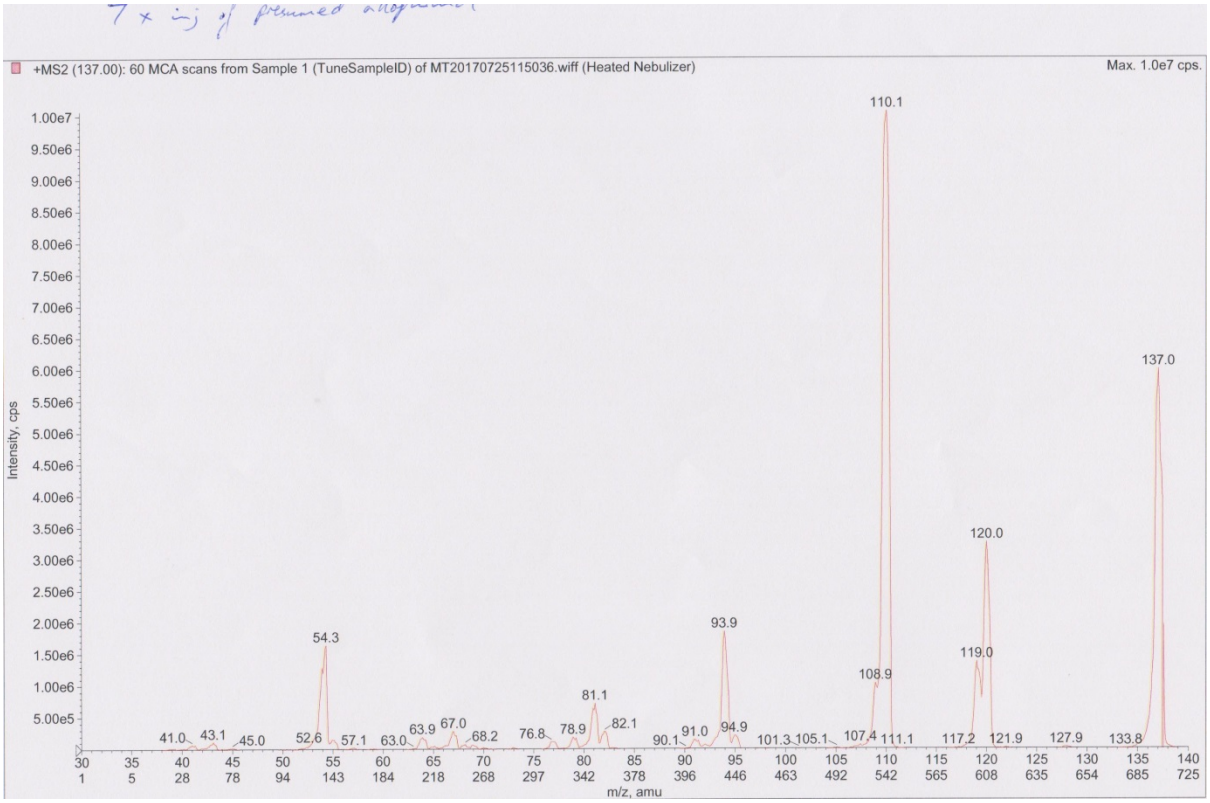


Figure 5: Allopurinol Product Ion Spectrum

It can be seen that there are several significant fragments from *m/z* 137. An MRM method was established, utilising the transitions from *m/z* 137 to *m/z* 110 and *m/z* 94. A piece of normal printer paper was cut into pieces measuring approximately 10 cm x 6 cm. Similar results were found from swabs from the side of the nose and forehead of the individual prescribed the medication as to those for ibrutinib. Clear responses to the presumed allopurinol were observed from both skin swabs (data not shown).

Conclusions

A qualitative method for the detection of two drugs was rapidly developed (the whole programme of work described took less than half an hour, much faster than performing this work using liquid sample introduction). The detection of the two drugs, ibrutinib and allopurinol, in sweat, was effected by means of a paper swab of the skin surface. The analysis required no solvents or glassware, and took a matter of seconds. Note that the raw drug was detected rather than a metabolite. This represents a fast method of investigating whether or not a compound can be detected; an assessment can then be made as to whether or not to fully optimise and validate a new method.

Although no certified reference materials were available, the fact that very similar findings were obtained from two different sources – the containers and the sweat – is strong evidence that the drugs were correctly identified, and highlights the versatility of the TEIS source.

Bibliography

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More information on the MSA TEIS is available here.

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