TIME OF FLIGHT MASS SPECTROMETRY AS AN INVALUABLE TOOL FOR SPECIFIC IDENTIFICATION OF REACTION AND DEGRADATION PRODUCTS WITHIN MEDICINAL CHEMISTRY

Michael McCullagh[1], Lena M. von Sydow[2]
[1]Waters Corporation, Simonsway, Manchester, M22 5PP, UK
[2]AstraZeneca, Gothenburg, S-43183 Mölndal, Sweden

INTRODUCTION

The use of reversed phase HPLC coupled to orthogonal acceleration time-of-flight mass spectrometry (oa-TOF-MS) is routinely chosen as the analysis method of choice within Medicinal Chemistry departments in the pharmaceutical industry. The continuous flow of compounds from synthetic chemists necessitates fast and specific analysis providing elemental composition confirmation of the target compound. The motive to this is to improve the quality of data and to minimize the risk of delivering the wrong compound for biological screening. The technique is also used for the determination of the identity of any by-products or degradation products present. This information is important to facilitate the interpretation of biological activity data and the understanding of reaction mechanisms.

Three different drug compounds from AstraZeneca have been studied: Omeprazole (active ingredient in Losec); Felodipine (active ingredient in Plendil) and Melagatran (prodrug in Exanta). Reaction and degradation products were identified using reversed phase HPLC coupled to oa-TOFMS. The results obtained using a new bench top oa-TOF technology (Waters® LCT Premier™) were compared with those acquired with classic oa-TOF technology (Waters LCT™). Two analytical laboratories were used for the comparison; the one at Medicinal Chemistry, AstraZeneca Mölndal, Sweden and the other at Waters Corporation, Manchester, Great Britain. The results obtained were extensive and only examples of the Felodipine and Xi-melagatran impurity profiling data will be presented.

Extracted mass chromatograms for the active drugs and impurities were used to allow peak integration and enable a response comparison to be made. The schematic of the oa-TOF with the analyser in W geometry is illustrated in Figure 1. The dynamic range enhancement (DRE) with this instrument operates routinely with an integral dual reference sprayer (LockSpray™). The mechanism by which DRE performs is represented schematically in Figure 2, and the DRE lens is shown in Figure 1. This novel approach allows the dynamic range to be increased without compromising resolution (>10000 FWHM) or sensitivity. During an acquisition the voltage applied to the DRE lens is switched rapidly between two values, resulting in high and low intensity data being produced for alternating ion accumulations. Typically 2% of the ion beam is transmitted to the analyser region during the reduced transmission state. The transmission switch allows any saturated high intensity data to be transparently replaced by low intensity data, which has been corrected for the transmission switch.

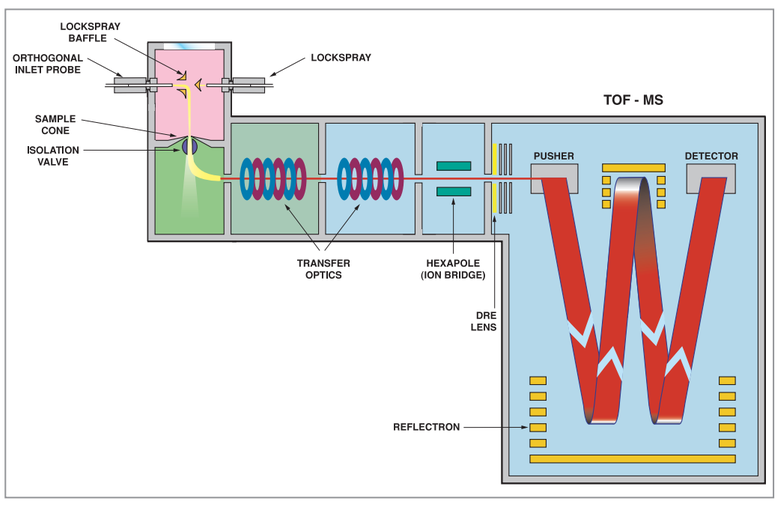

Figure 1. Oa-TOF schematic (W mode >10000 FWHM).

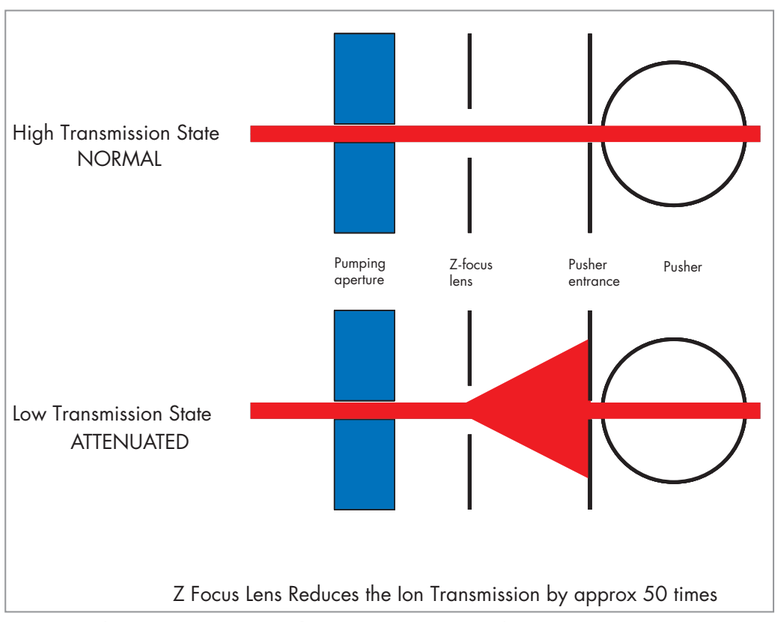

Figure 2. Schematic representation of DRE (Dynamic Range Enhancement).

EXPERIMENTAL

Instrumentation

System 1

Mass Spectrometer:	Waters® Micromass® LCT Premier™
HPLC:	Waters Alliance® HT 2795

System 2

Mass Spectrometer:	Waters Micromass LCT™
HPLC:	Agilent 1100 System

HPLC Conditions

Column:	Atlantis® C_{18} (150 mm x 2.1 mm, 3.5 µm particle size)
Column temperature:	20 °C
Flow:	0.2 mL/min
Mobile phase:	A: H_2O (0.1 % Formic Acid) B: MeCN (0.1 % Formic Acid)
Gradient:	0–0.50 min: 2% B 0.5–10 min: 80% B 10–20 min: 80% B 21 min: 2% B 25 min: 2% B

MS Conditions

Ionization mode:	ESI+ at 3 kV
Sample cone voltage:	30V (LCT) and 100V (LCT Premier).
Reference mass:	Leucine enkephalin, [M+H]+ =556.2771
Acquisition Parameters:	100–1000 m/z 1 spectrum/second 0.1 second inter scan delay
Resolution:	5000 FWHM (V mode) 10000 FWHM (W mode)

RESULTS

The DRE TIC obtained for the analysis of Xi-melagatran (500 µM) is presented in Figure 3 with the exact extracted mass chromatograms for A: impurity 1 (hydroxy-Melagatran), C: impurity 2 (Melagatran amide) and the prodrug B: Xi-melagatran. The corresponding area response obtained for Xi-melagatran impurity 2 at prodrug concentrations of 50 µM, 250 µM and 500 µM are shown in Figure 4. The exact mass spectra and mass measurement error obtained for impurity 2 at each respective concentration is illustrated in Figure 5. From Figure 6 the area response obtained for Xi-melagatran impurity 1 can be seen where analysis was performed using classic oa-TOF technology. In Figures 7 and 8 the corresponding area responses obtained for the prodrug Xi-melagatran, impurity 1 and impurity 2 are shown from the classic oa-TOF and the new oa-TOF, respectively. The impurity profile for Felodipine is illustrated in Figure 9 and the corresponding exact mass measurement errors for the impurities and active identified are shown in Table 1.

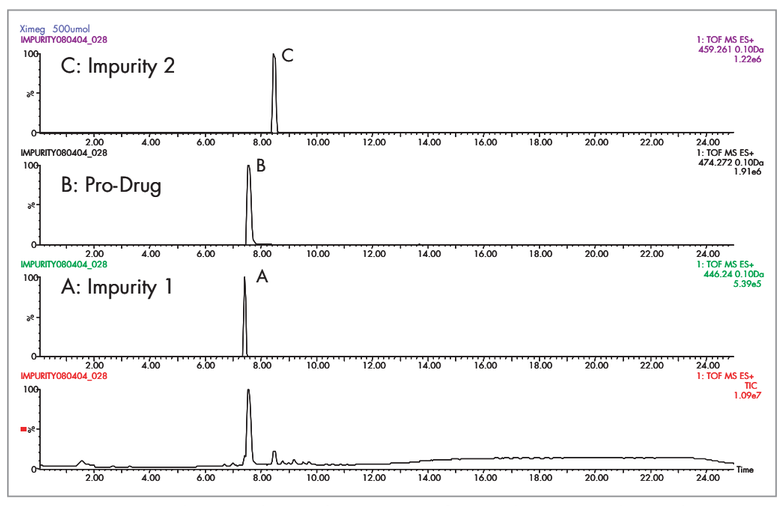

Figure 3. DRE TIC and extracted mass chromatograms for Xi-melagatran prodrug and impurities 1 and 2 at a concentration of 500 µM for prodrug.

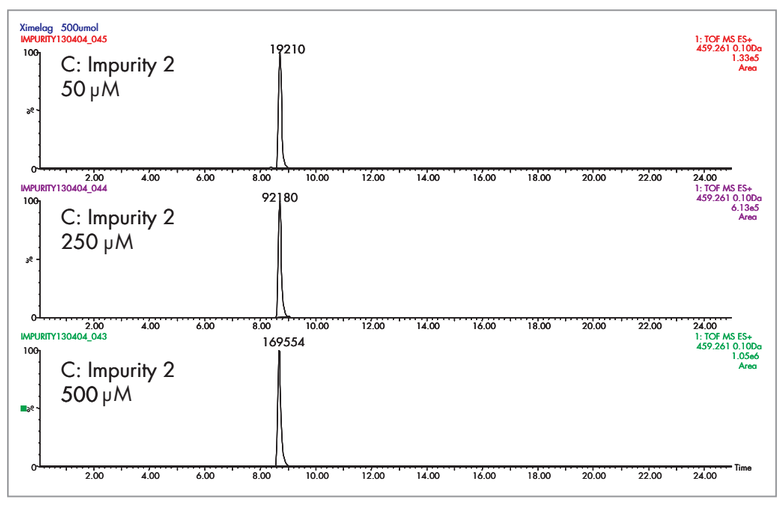

Figure 4. DRE extracted mass chromatograms for Xi-melagatran impurity 2 at Xi-melagatran prodrug concentrations of 50 µM, 250 µM and 500 µM, respectively.

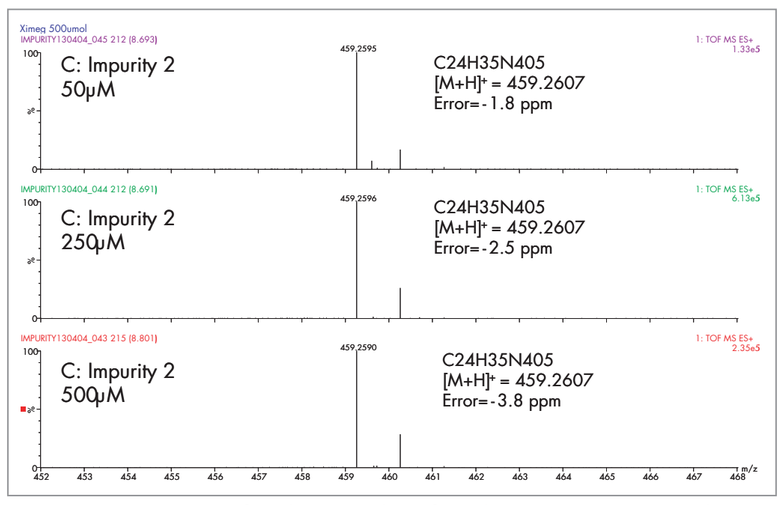

Figure 5. Exact mass spectra from the DRE analysis of Xi-melagatran impurity 2 at Xi-melagatran prodrug concentrations of 50 µM, 250 µM and 500 µM respectively.

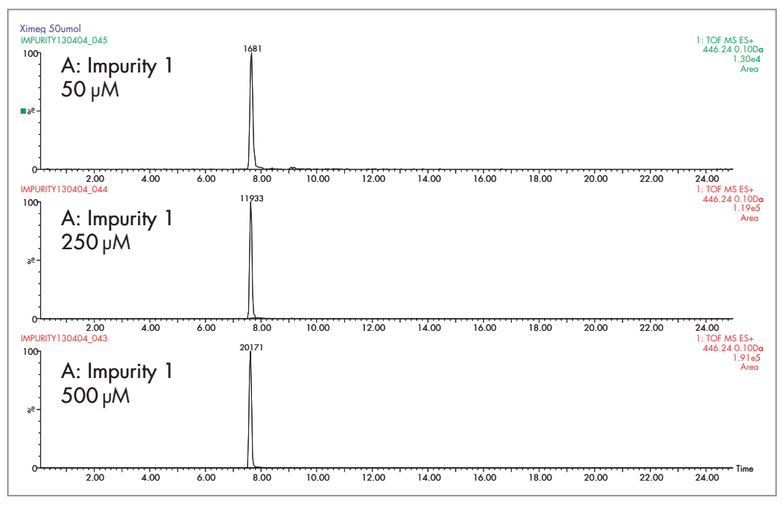

Figure 6. Classic oa-TOF extracted mass chromatograms for Xi-melagatran impurity 2 at concentrations of prodrug Xi-melagatran, 50 µM, 250 µM and 500 µM.

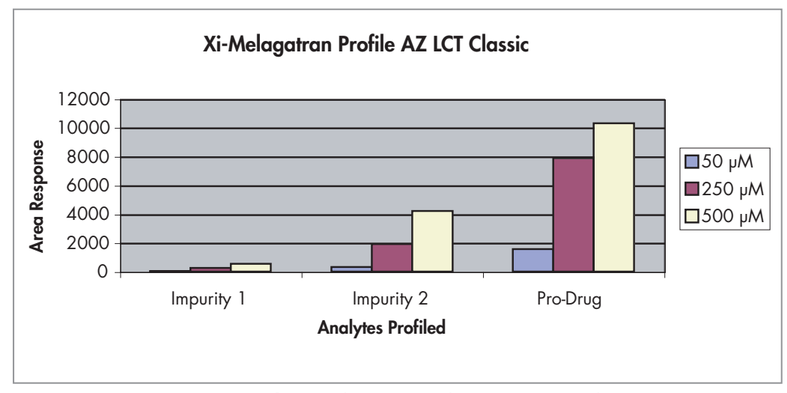

Figure 7. AZ LCT area response for Xi-melagatran prodrug, impurity 1and impurity 2 at 50 µM, 250 µM and 500 µM (concentration of prodrug).

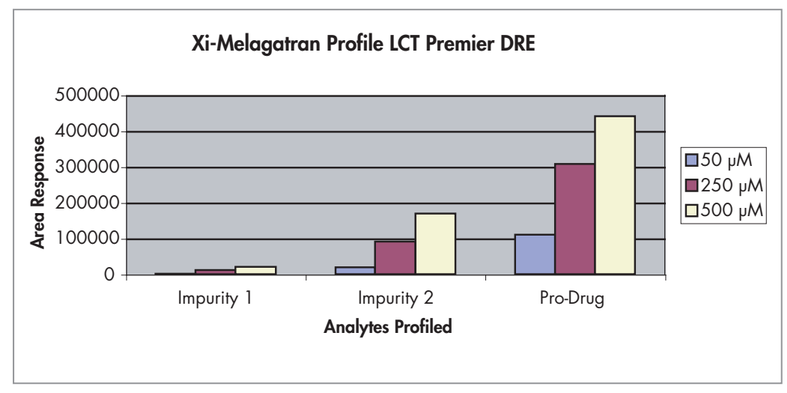

Figure 8. LCT premier area response for Xi-melagatran prodrug, impurity 1 and impurity 2 at 50 µmol, 250 µmol and 500 µmol (concentration of prodrug).

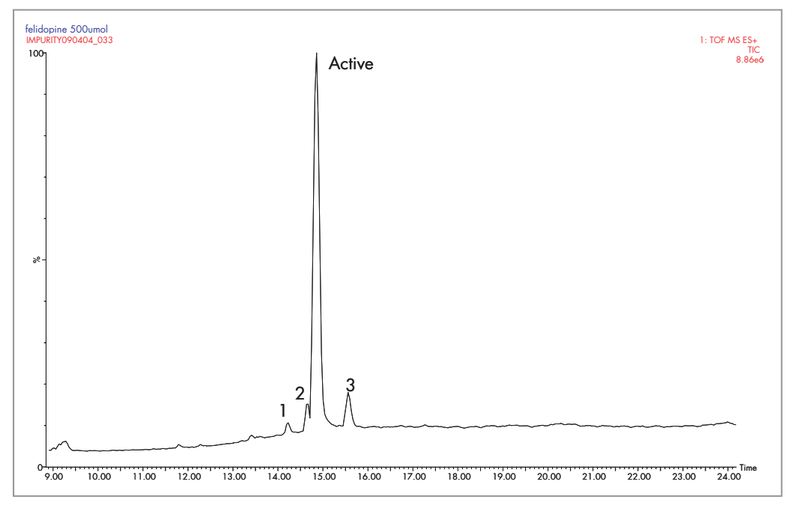

Figure 9. DRE TIC for Felodipine active (Plendil) and impurities 1, 2 and 3.

Elemental Composition	Drug Concentration		
	50 µM	250 µM	500 µM
C18H20Cl2NO4 384.0769	4.6 ppm 384.0787	-2.7 ppm 384.0759	1.2 ppm 384.0787
C18H18Cl2NO4 382.0613	1.6 ppm 382.0619	-4.9 ppm 382.0594	-3.1 ppm 382.0601
C17H16Cl2NO3 352.0507	2.2 ppm 352.0515	-2.3 ppm 352.0499	-4.0 ppm 352.0493
C16H14Cl2NO3	11.6 ppm 338.0390	0.7 ppm 338.0353	0.0 ppm 338.0351
RMS Error	7.26 ppm	3.047 ppm	2.60 ppm

Table 1. Exact mass measurement errors for the impurity profile of Felodipine using DRE.

DISCUSSION

Previously LC-TOF instruments have been restricted by limited dynamic range. This led to rather complicated determination of which scans to use for accurate mass determinations. The results from the present study show that LC-TOF in DRE mode gives normal spectra and chromatograms that include high intensity peaks, which would have normally been saturated. Typically impurity profiling data would be acquired using a concentration of 500 µM, based on the molecular weight of the active component. The experiments undertaken were required to show that working concentrations of the active component could be reduced to 50 µM.

As can be seen the increase in sensitivity using the LCT Premier compared to the LCT classic is more than ten times. Furthermore, the observed increase in response produces improved ion statistics and therefore improved exact mass measurement for low level impurities such as those identified. DRE extends the working dynamic range enabling exact mass measurement to be obtained at the high response levels obtained for the active compounds of the drugs analysed. In Figure 5, impurity 2 is mass measured with –2.5 ppm error at as high as 613000 counts per second. Felodipine was also profiled successfully; where RMS errors of less than 3ppm were obtained for the impurities and active at 250 µM and 500 µM.

CONCLUSIONS

- Mass measurement errors of <3ppm were obtained routinely and consistently when using the W mode at a resolution of >10 000 (FWHM).
- The highly specific nature of exact mass measurement enables elemental compositions to be easily and rapidly determined and therefore confidence in producing the correct identification of the screened substances as well as the impurities formed.
- DRE extends the dynamic range for which exact mass measurement can be made to 4 orders.
- DRE also offers the possibility of simultaneous accurate mass determinations of high concentration substances in the same chromatogram as low concentration by-products.
- Determination of accurate mass using DRE is much simplified compared to non-DRE mode; one scan at the apex of the peak is sufficient in DRE mode, whereas only scans at the slope of the peak can be used in non-DRE mode because of saturation at much lower concentrations.
- The LCT Premier oa-TOF with integral LockSpray and independent reference mass acquisition enables the routine acquisition of highly specific data.
- Greater than ten times more sensitivity was achieved using the LCT Premier, producing better ion statistics for low level impurities and hence improved exact mass Measurement.

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