HIGH THROUGHPUT IDENTIFICATION OF REACTION AND DEGRADATION PRODUCTS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY AND TIME OF FLIGHT MASS SPECTROMETRY

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INTRODUCTION

Ultra performance liquid chromatography (UPLC™) coupled to orthogonal acceleration time-of-flight mass spectrometry (oa-TOF-MS) enables increased sample throughput and selectivity. Reversed phase HPLC has previously been selected as the routine analysis method of choice within the pharmaceutical industry. Technological advances have provided improved separation and detection of reaction and degradation products supplying new information to the analytical and synthetic chemists. In the case of mass spectrometers not capable of performing MS/MS, the chromatographic resolution produced using UPLC™ also facilitates a new dimension to data acquisition. The acquisition of pure "in-source" CID (collision induced dissociation) accurate mass spectra is feasible, where the selectivity required is brought about by chromatography rather than mass selection using a mass spectrometer. This improves the ability to obtain structural information when MS/MS functionality is unavailable.

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 present. This information is essential to facilitate the interpretation of biological activity data.

Three different drug compounds from AstraZeneca have been studied: Omeprazole (active ingredient in Losec); Felodipine (active ingredient in Plendil) and Melagatran (active ingredient in Exanta). Reaction and degradation products were identified using reversed phase HPLC and UPLC™ coupled to oa-TOF-MS. Data illustrating analysis time reduction with improved chromatographic resolution will be presented. In addition using oa-TOF-MS, pure CID accurate mass fragmentation spectra will be shown.

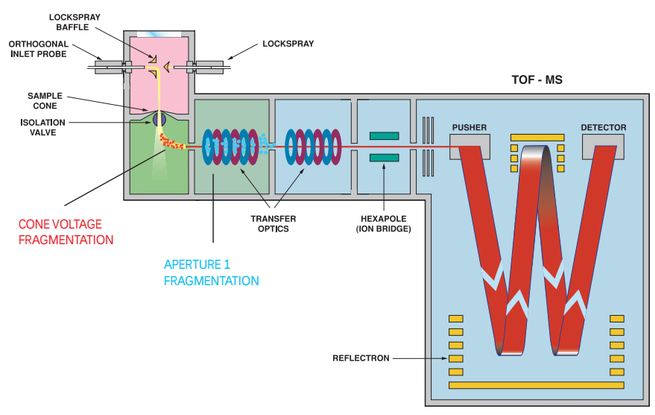

Figure 1. Oa-TOF schematic (W mode > 10000 FWHM) illustrating two regions in which "in-source" CID can be implemented.

EXPERIMENTAL

Mass Spectrometer: Waters Micromass LCT Premier™
Ionization Mode: ESI+ at 3 kV; Sample cone voltage: 35V
Reference mass: Leucine enkephalin, $[M+H]^+$ =556.2771
Acquisition Parameters: 100-1000 m/z;
HPLC: 1 second/spectrum; 0.1 second inter acquisition delay
UPLC™:0.15 second/spectrum; 0.05 second inter acquisition delay
Resolution = >5000 FWHM (V mode) and 10000 FWHM (W mode)

HPLC: Waters® Alliance®HT 2795
Column: Atlantis® C_{18} 150 mm x 2.1 mm, 3.5 mm particle size
Column temperature: 20°C Flow: 0.2 mL/min
Mobile phase: MeCN (0.1 % Formic Acid) (B): H2O (0.1 % Formic Acid) (A)
Gradient: 0 min: 2% B; 10 min: 80% B; 10 - 20 min: 80% B; 21 min: 2% B; 25 min: 2% B.
Analytes: Felodipine and Xi-melagatran

System: Waters ACQUITY UPLC™
Column: Waters ACQUITY UPLC™ BEH C_{18} (100 mm x 2.1 mm, 1.7 mm particle size)
Mobile phase I: MeCN (B) : H2O (0.1% HCOOH) (A)
Mobile phase II: MeCN (B) : H2O (Ammonium Formate [pH 9]) (A)
Gradient: 0 min 15% B, 4.0 min 85% B, 9.0 min 85% B, 9.1 min 15% B, 10.0 min 15% B
Column temperature: 40°C Flow: 0.6 mL/min
Analytes I: Felodipine and Xi-melagatran
Analyte II: Omeprazole

RESULTS

In Figure 2 the TIC for Felodipine active (Plendil) and impurities 1, 2 and 3 acquired using HPLC/MS is presented and the corresponding UPLC™/MS BPI and extracted mass chromatograms are shown in Figure 3. From Figure 4 the BPI and extracted mass chromatograms for Xi-melagatran prodrug and impurities 1 and 2 acquired using HPLC/MS can be seen, where as Figure 5 contains the UPLC™/MS data. The single compound accurate mass in-source CID spectrum for Xi-melagatran acquired with oa-TOF/UPLC™ is illustrated in Figure 6. The extracted mass chromatograms for Omeprazole active (Losec) and impurities 1, 2, 3 and 4 acquired using UPLC™/MS are presented in Figure 7. Figure 8 shows the single compound accurate mass in-source CID spectrum for Omeprazole. In Figure 9 the single compound accurate in-source mass CID spectrum for Omeprazole degradation products 2 and 3 acquired with oa-TOF/UPLC™ are shown. The HPLC/UPLC™ retention time and resolution comparisons for the profiling of Felodipine and Xi-melagatran are shown in Tables 1 and 2 respectively.

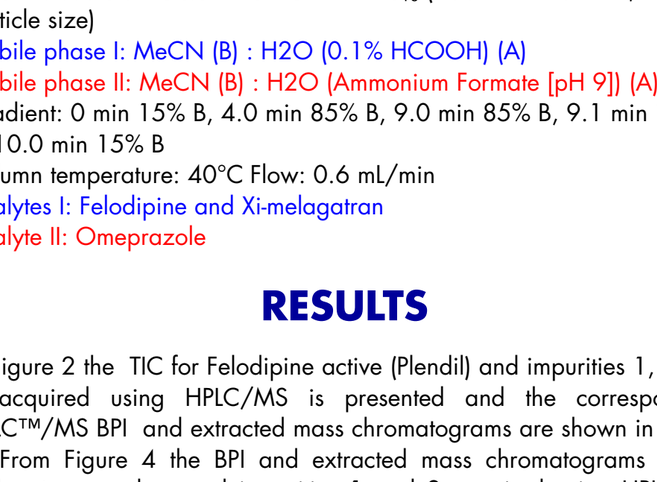

Figure 2. TIC for Felodipine active (Plendil) and impurities 1, 2 and 3 acquired using HPLC/MS.

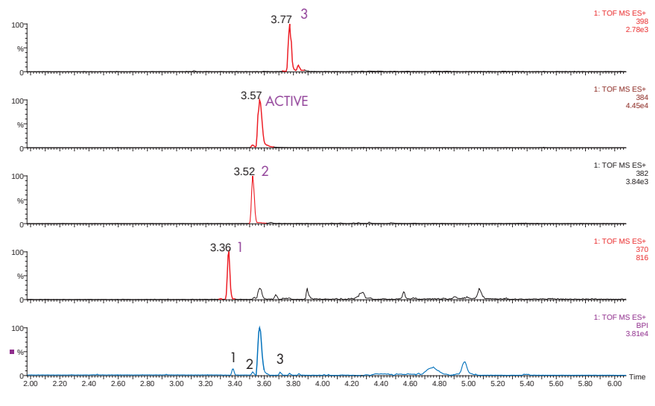

Figure 3. BPI for Felodipine active (Plendil) and extracted mass chromatograms for impurities 1, 2 and 3 acquired using UPLC™/MS.

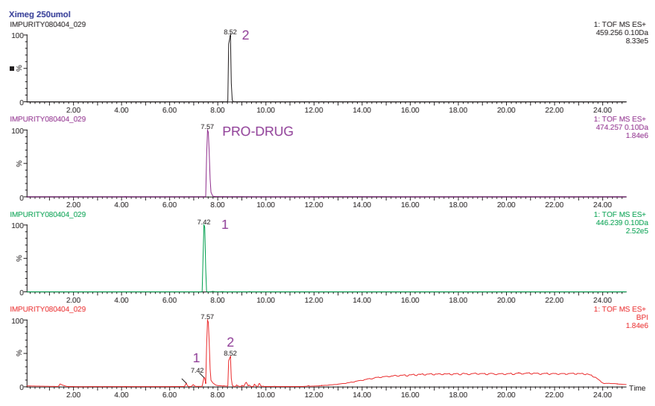

Figure 4. BPI and extracted mass chromatograms for Xi-melagatran prodrug (Exanta) and impurities 1 and 2 acquired using HPLC/MS.

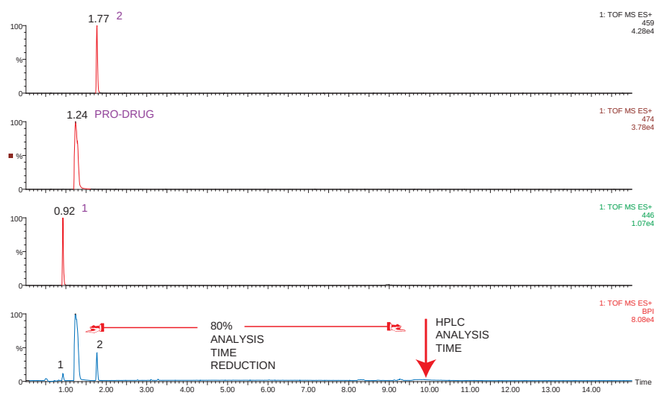

Figure 5. BPI and extracted mass chromatograms for Xi-melagatran prodrug (Exanta) and impurities 1 and 2 acquired using UPLC™/MS.

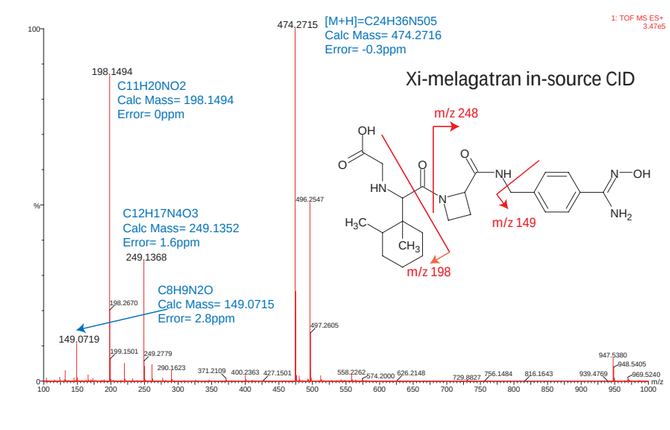

Figure 6. Single compound accurate mass in-source CID spectrum for Xi-melagatran acquired with oa-TOF/UPLC™.

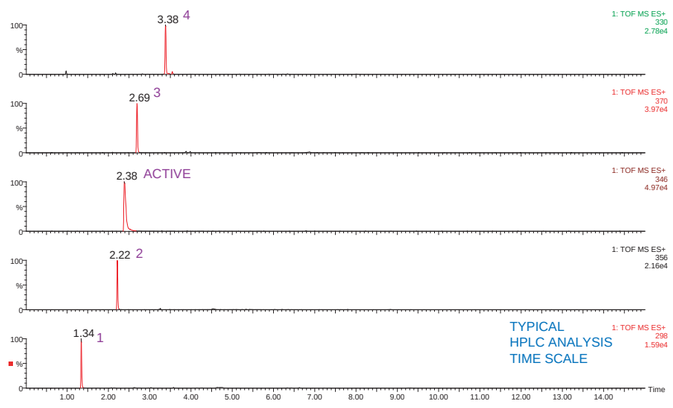

Figure 7. Extracted mass chromatograms for Omeprazole active (Losec) and impurities 1, 2, 3 and 4 acquired using UPLC™/MS.

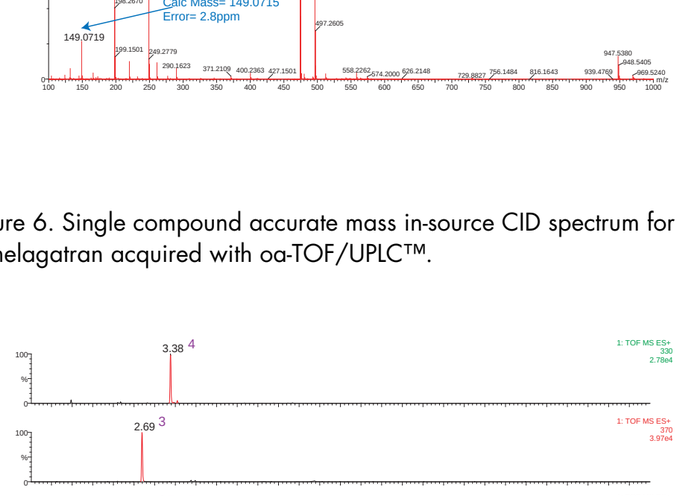

Figure 8. Single compound accurate in-source mass CID spectrum for Omeprazole acquired with oa-TOF/UPLC™.

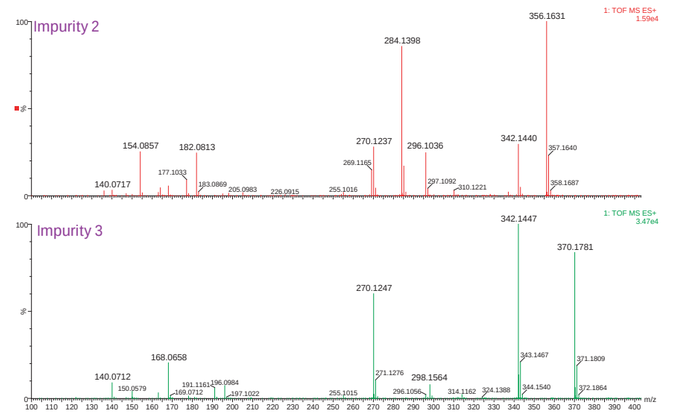

Figure 9. Single compound accurate in-source mass CID spectra for Omeprazole degradation products 2 and 3 acquired with oa-TOF/UPLC™.

Table 1 Table to show the resolution of Felodipine using UPLC™ and HPLC/MS.

Peak No	m/z	HPLC RT	UPLC RT	RS	RS	RS
1	370	14.23	3.36	1.73, **3.20**		
2	382	14.23	3.52		0.42, **0.58**	
3ACT	384	14.85	3.57			1.27, **2.22**
4	398	15.55	3.77			

Table 2 Table to show the resolution of Xi-melagatran using UPLC™ and HPLC/MS.

Peak No	m/z	HPLC RT	UPLC RT	RS	RS
1	446	7.42	0.92	0.54, **1.03**	
2ACT	474	7.57	1.24		2.84, **3.65**
3	459	8.45	1.77		

DISCUSSION

In a previous study the reaction and degradation products of three drug compounds from AstraZeneca were identified using reverse HPLC coupled to oa-TOF-MS. The compounds of interest were: Omeprazole (active ingredient in Losec); Felodipine (active ingredient in Plendil) and Melagatran (prodrug in Exanta).[1] The results from this study have been compared to those obtained using UPLC™ coupled to oa-TOF-MS. In Figures 2 and 3 the HPLC and UPLC™/MS degradation profiling results are shown for Felodipine. It can be seen that there is a significant reduction in analysis time, for impurity 3, the elution time has been reduced by 75%. In Table 1 the resolution obtained between the peaks of interest identified in Felodipine are listed. In every case analysis time is reduced by 75% and chromatographic resolution is improved. In Figures 4 and 5 the HPLC and UPLC™/MS degradation profiling results are shown for Xi-melagatran. It is shown that there is a significant reduction in analysis time, for the pro-drug, the elution time has been reduced from 7.57 minutes using HPLC to 1.24 minutes with UPLC™. This corresponds to an analysis time saving of greater than 80%. In Table 2 the resolution obtained between the peaks of interest identified in Xi-melagatran are listed. In every case analysis time is reduced by 80% and chromatographic resolution is improved. In Figure 7 the UPLC™/MS degradation profile of Omeprazole is presented. The extracted ion chromatograms of four degradation products and the active compound are shown. The chromatographic peaks widths at base were 3.6 seconds. This improved chromatographic resolution can now be used to add a further dimension to MS acquisition, where MS/MS functionality is not available. Utilising in-source CID to provide structural information can provide valuable information. However this technique does not have the functional selectivity of MS/MS, to provide single component spectra where two compounds are co-eluting. High resolution chromatography increases the probability of obtaining single component peaks. This enables single component in-source CID spectra to be acquired. Even with fast analysis times chromatographic resolution has been improved, using a shallower gradient will further increase the probability of single component peaks to be obtained. In Figure 1 it is illustrated that there are two routes to obtaining in-source CID fragmentation data. Fragmentation can be achieved by simply increasing the cone voltage or by increasing a lens voltage in the transfer region. This allows two CID functionalities to be applied simultaneously enabling dual stage pseudo MS/MS data to be generated. UPLC™ combined with accurate mass measurement can be used to provide specific structural information. Examples of the accurate mass spectra obtained for Xi-melagatran and Omeprazole are shown in Figures 6 and 8. In both cases it has been possible to determine the elemental composition of the fragments obtained with less than 3ppm error, just by averaging the whole chromatographic peak. For all three drug compounds profiled single component CID spectra were acquired, allowing common fragments and hence impurity structure to be proposed. This is illustrated in Figure 9, where the fragmentation relationship and hence structural similarity to the parent compound can be visualised.

CONCLUSIONS

- UPLC™ provides selectivity to add another dimension to MS data acquisition.
- UPLC™ provides a route to single component CID fragmentation spectra in a short time frame.
- Specific structural information is achieved.
- Confidence is provided by accurate mass measurement within 3ppm error.
- Elemental composition of fragments provide rapid identification.
- Felodipine analysis time reduced from 16 minutes to 4 minutes.
- Xi-melagatran analysis time reduced from 9 minutes to 2 minutes.
- Omeprazole analysis time completed in 4 minutes.
- Actives and impurity resolution increased.
- Analysis time reduced by at least 75% using a generic method.
- Improved chromatographic resolution has been obtained routinely.

References

1. Time of Flight Mass Spectrometry as an Invaluable Tool for Specific Identification of Reaction and Degredation Products Within Medicinal Chemistry. (Waters Application Note 720001068 EN).

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