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Faster compound analysis by mass spectrometry — the ToF revolution☆

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Abstract

The introduction of the orthogonal Time of Flight (ToF) analyser has enabled ToF to be applied to continuous ionisation sources. In this technique, pulses of ions are ejected orthogonally from the main ion beam. This paper describes our development of techniques utilising the advantages of orthogonal-ToF for the analysis of small molecules especially drug substances. The fast scanning ability of the ToF enables its use with fast chromatographic procedures and can also be utilised to enable parallel detection of multiple LC columns. The medium resolution giving accurate mass measurement is utilised for faster and more reliable structural determination. These techniques are being used daily in our laboratories and some examples are shown. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Mass spectrometry; Accurate mass measurement; Drug substance analysis; Parallel HPLC analysis

1. Introduction

The requirement within the pharmaceutical industry to discover and develop new drugs more rapidly has led to an explosion in the number of samples requiring analysis. With the introduction of automated activity screening (100000 per diem!) for biological testing and combinatorial chemistry technologies to generate many more compounds, analysis to confirm structures becomes a rate limiting step in the drug development process. A recent estimate of the number of samples requiring analysis showed an increase from 8000 p.a. in 1995 to 200000 in 1999 [1]. There are a number of ways of meeting this need.

Firstly buy more mass spectrometers. This was an approach used in some areas (such as biofluids analysis) and laboratories where tens of instruments carrying out automated routine analysis are found. However, this creates difficult resourcing issues.

Secondly, carry out faster analysis [2]. This is an area where the ToF starts to make an impact with its fast scanning and ability to reproduce spectra.

The Time of Flight mass spectrometer (ToF) was developed in the 1950s as a fast scanning analyser [3]. At this time its advantages were its

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speed (a few microseconds per scan), its ability to record a complete spectrum for each accelerating pulse, thus accurately recording the relative intensities of the ions, and finally its dependence on the electronics for its performance, rather than accurate machining of parts to ensure mechanical alignment. Its main disadvantage at this time was its low resolution (ca. 500) but a more significant disadvantage was its complexity when using online continuous ionisation sources (which includes most common sources such as EI, CI, electrospray, etc.). In this process ions exiting from the source had to be gated and also receive equivalent acceleration to prevent spread and loss of resolution. These factors were the main reasons for the poor resolution especially the spread of energy for ions in the beam.

Workers in the later 1980s and early 1990s readdressed the problem of using ToF analysers with continuous ionisation sources. Although there is a kinetic energy distribution along the axis of the ionisation beam, there is almost no distribution orthogonally to that beam. Thus by accelerating ions orthogonally a tight energy distribution can be obtained. The accelerating voltage can also be pulsed to suit the ToF analyser. Such a system was described for a GC/MS using an EI source in 1989 [4]. Shortly after other workers described the combination of an electrospray source with an orthogonal ToF (oa-ToF) [5]. The oa-ToF analyser was also utilised in a number of hybrid instruments, of which the most popular has proved to be the Quadrupole-ToF first reported in 1996 [6].

The current generation of oa-ToF spectrometers display medium resolution, fast scanning and a high degree of sensitivity. Although developed with bioanalytical applications in mind we realised that these attributes would also have an impact on our analytical work dealing with the spectrometric analysis of small molecules. This paper describes the applications we have developed since 1997 for such molecules and the improvements in quality and throughput we have achieved.

2. Experimental

The attributes of the oa-ToF analyser which have had such an impact on our work with small molecules are

The fast scanning ability;
the reproducible spectra;
the sensitivity;
the medium resolution and accurate mass determination.

Examplars are given illustrating these various attributes.

2.1. Fast scanning spectra

Chromatography was carried out using a model 1090 hplc system (Agilent Technologies, Stockport, UK) fitted with a Luna C18 column (3 μ packing, 50 × 2.0 mm). The column was held at 40°C [2]. Solvent A was 0.05 M ammonium acetate in water adjusted to pH 4.5 with acetic acid; solvent B was acetonitrile. A gradient from 100% A to 40% A was run over 5 min at a flow rate of 600 μl min^{-1}. Mass spectrometry was carried out using a Micromass Q-ToF mass spectrometer fitted with an orthogonal electrospray source (Micromass UK Ltd., Wythenshawe, UK). Spectra were acquired over a mass range of 100–1000 u.

2.2. Reproducible spectra

Gas chromatography mass spectrometry was carried out using a DB-5MS column (30 m × 0.25 μm) programmed from 40 to 300°C at 10°C min^{-1}. Low resolution mass spectra were obtained using electron ionisation and a Micromass Trio-2 gas chromatograph single quadrupole mass spectrometer. ToF spectra were acquired using electron ionisation and a Micromass GC-T oa-ToF mass spectrometer.

2.3. Parallel analysis

HPLC analysis were carried out using a Waters 600 pump feeding eight columns mounted on a Gilson 215 multiple injector autosampler [7]. Samples were held in polypropylene microtitre

plates and eight simultaneous injections made (15 μl each). The columns (Zorbaz SB C18 2.1 × 30 mm) each received a flow of 200 μl min^{-1}. The columns were attached to the eight ports of the MUX™ interface of a Micromass LC-T oa-ToF mass spectrometer and analysed in sequence with a dwell time of 0.1 s. followed by a switching interval of 0.1 s.

2.4. Accurate mass measurement

HPLC was carried out using a Symmetry C18 column (250 × 4.6 mm) and a HP1100 hplc system. This was attached to the electrospray source of an Applied Biosystems Mariner oa-ToF mass spectrometer. A solution of Jeffamin M-series was added post column using a 'T-piece' to act as a lock mass.

3. Results

When using a scanning instrument such as a quadrupole for GC/MS analysis the time for a scan becomes critical using capillary columns with narrow GC peaks. The concentration of compound in the source will change rapidly as the material elutes and within a scan the intensity of ions at the low mass end will be distorted relative to the higher field ions (Fig. 1). The pusher for the oa-ToF will isolate a packet of ions in the ion beam at an instant and hence the spectra more closely retain the relative abundances of ions representative of the population in the source (Fig. 2). This advantage has been noted since the earliest ToF experiments [3] and also applies to work with fast capillary column liquid chromatography mass spectrometry.

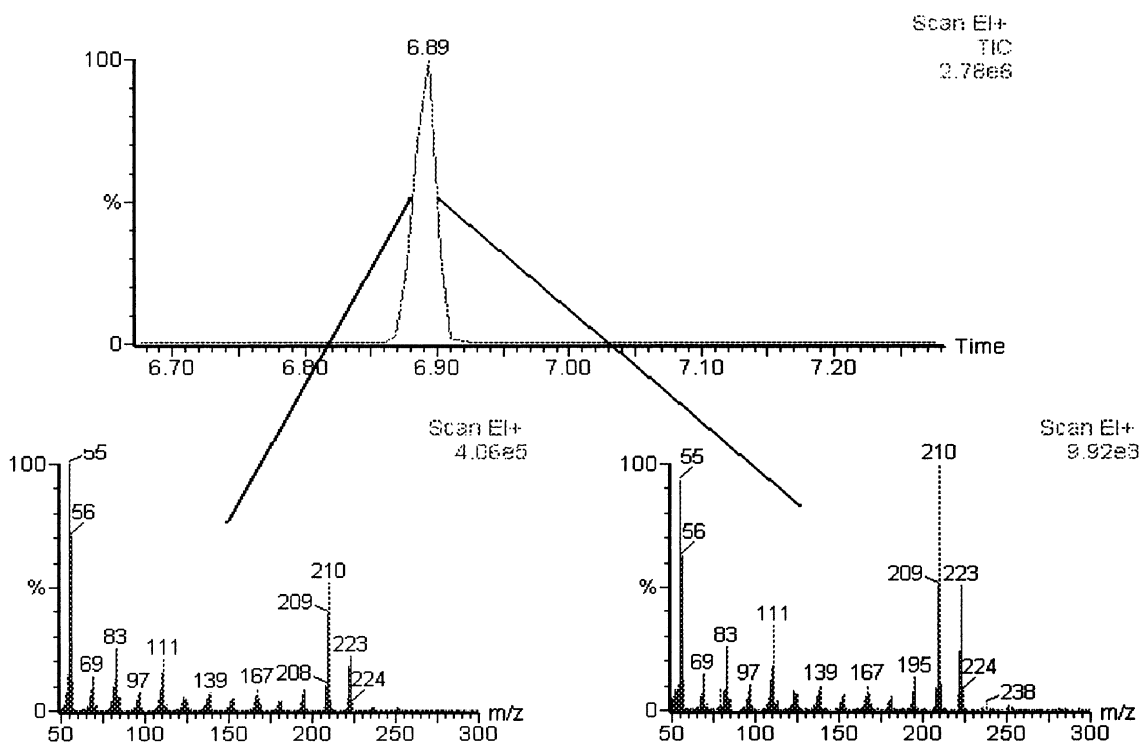


Fig. 1. Comparison of EI spectra obtained on the rising and falling edges of a capillary GC/MS peak using a quadrupole analyser.

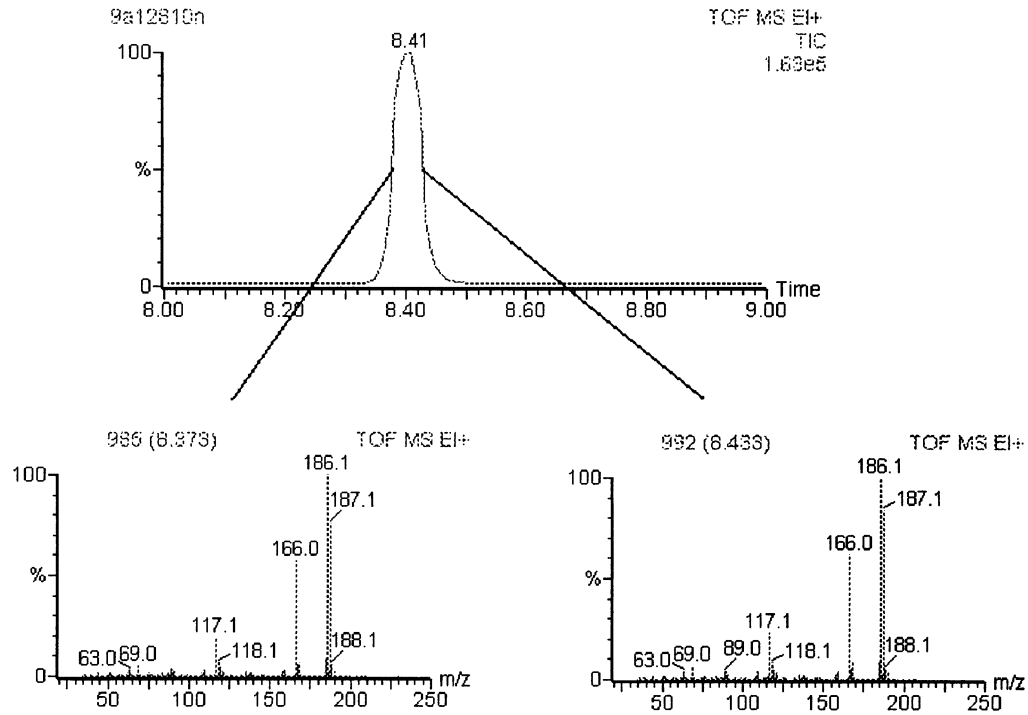


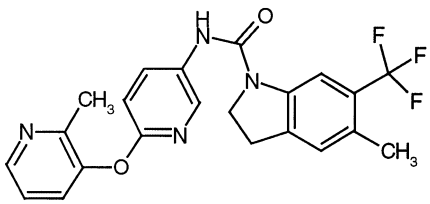
Fig. 2. Comparison of EI spectra obtained on the rising and falling edges of a capillary GC/MS peak using a ToF analyser.

Fast scanning also enables the use of parallel analysis, a concept developed in the last two years. By coupling multiple columns to a modified inlet system sequential spectra can be obtained from upto eight LC eluents [7]. This system, using an eight way autosampler and fast chromatographic columns (Fig. 3), enables LC/MS analysis of a 96-well titre plate to be carried out in less than one hour when earlier it required almost a whole working day. The simple mechanical interface ('MUX™' Micromass Ltd., Manchester, UK, Fig. 4) effectively blocks seven eluent sprays allowing only one to be sampled. After a period of data collection the device steps to the next position. Once all eight have been sampled then the device is returned to stage 1. The data are collected into separate files. Typical sampling times are 0.1 s with a 0.1 s delay for each step, hence a cycle time of 1.6 s is possible. Despite the possible risk of streams 'mixing' and adjacent beams cross contaminating each other this has not been observed in practice and clean spectra and chromatograms are obtained for all eight samples.

Currently research grade oa-ToF spectrometers are giving about 10000 resolution, which for most small molecule work gives mass accuracies of < 10 ppm [8]. Accurate mass measurement is performed by adding a suitable reference compound through a T-piece to the column eluent [9]. Using the reference known mass as a lock mass is sufficient to give an accurate mass for the unknown. In our experience, this is sufficient to generate an empirical formula for most compounds as the chemical history is known enabling the analyst to reduce considerably the number and type of hetero-atoms to be expected. Simple modifications by degradation or metabolism can also be studied. The combination of good sensitivity and mass accuracy mean that structures of minor impurities in drug substance, for example, can be established; although in cases where it is possible, complementary analysis using other spectroscopic techniques such as NMR is essential to produce definitive structures. As an example a drug substance was analysed by LC/MS to provide accurate masses of minor impurities. The

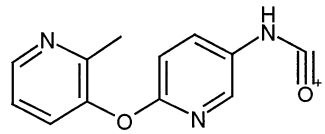
results are shown in Table 1. Accurate masses were obtained on components with a UV response of $< 0.001\%$ of the main peak.

Hybrid instruments, especially Qq-oaToF, give the added advantage of accurate mass measurement of product ions. In these experiments a precursor ion is selected by Q1 and fragmented in the collision cell q2. This is accomplished using argon as the collision gas. The collision cell is tuned (gas pressure and energy) to transmit 5–10% of the precursor ion. This is then used as a lock mass (its accurate mass being known) in the ToF scan to generate accurate masses for the fragment ions [8,9]. An example of this approach is the analysis [8] of SB-243213, which is unusual inasmuch it has isobaric fragments about the carbonyl moiety (I).

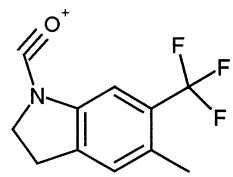


I

Fragmentation generated principally an ion of nominal mass 228, which may be due to the isobars (II, III).



II



III

Accurate mass measurement gave a mass of 228.0750. Isobar II, $C_{12}H_{10}N_3O_2$, requires 228.0773 whilst isobar III, $C_{11}H_9F_3NO$, requires 228.0636. Thus, fragmentation gives predominantly isobar II with a difference of only 0.0023 u (about 10 ppm). Although a resolution of 17000

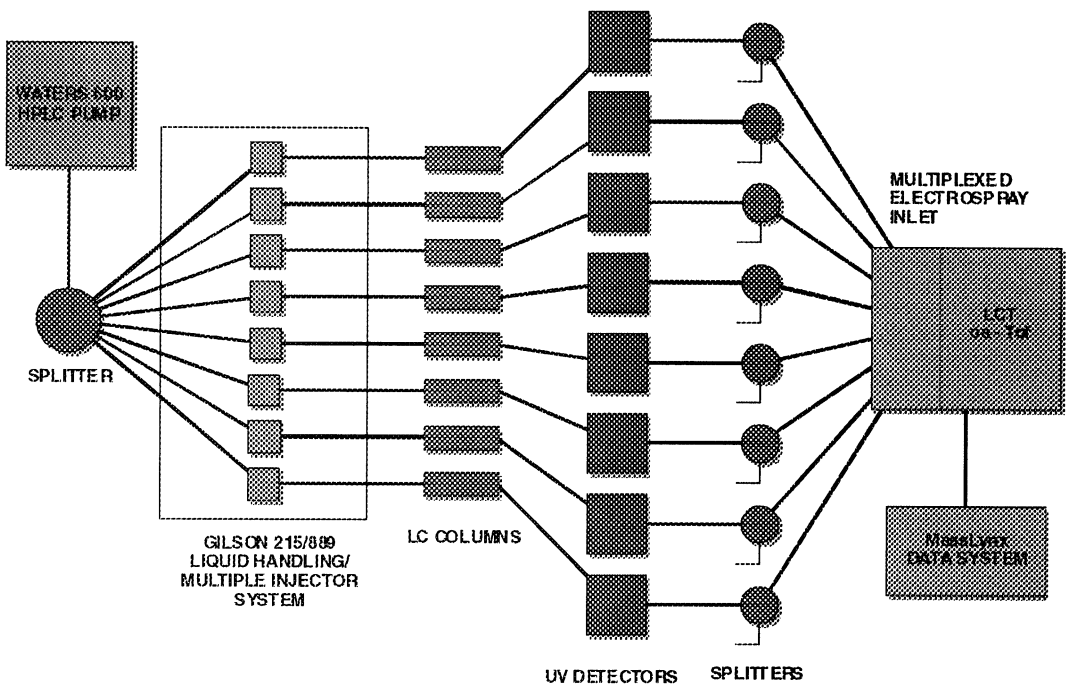


Fig. 3. Layout of an 8-way parallel LC/MS system. Diagram with permission of Micromass UK Ltd.

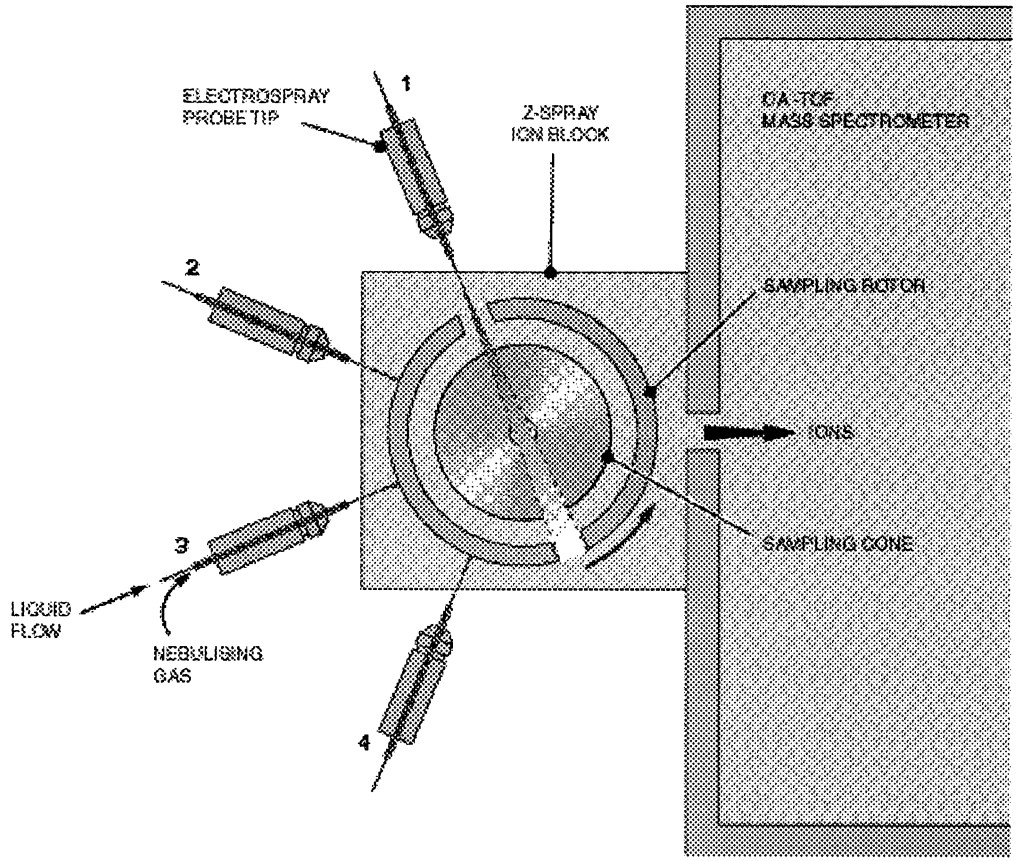


Fig. 4. Plan of the multi-inlet interface (MUX™) for a LC/ToF mass spectrometer. Diagram with permission of Micromass UK Ltd.

is required to separate II from III, a mixture of both would show a drift towards the lower mass.

There are some drawbacks to the methodology especially the need to add reference through a T-piece. The additional dead volume can be detrimental to the chromatography, especially working with micro or capillary columns. Also, admixing the reference and analyte can cause problems with suppression of ionisation of one by the other. These difficulties can be overcome by using a modified 'MUX™' system for accurate mass measurement [10].

4. Summary

The various attributes of the modern ToF mass spectrometer have had a major impact on the way we carry out mass spectrometry.

The fast scanning has allowed quality mass spectra to be obtained with fast chromatographic systems.

The reproducible spectra independent of changes in source pressure allow the analysis of narrow peaks from capillary GC and narrow bore HPLC columns.

The medium resolution enables accurate mass measurement on weak peaks due to minor impurities.

The combination with a quadrupole first stage enables accurate mass measurement of fragment ions from a MS/MS experiment improving the certainty of identification.

Table 1
Accurate mass measurements obtained for a number of minor impurities identified by LC/MS using an orthogonal ToF mass spectrometer

Component	Height (%) base peak	Formula	Mass calculated	Error observed, mmu
Drug substance	100	$C_{23}H_{33}N_4O_4$	429.2502	−0.5
Impurity A	0.010	$C_{24}H_{33}N_4O_5$	457.2451	0.7
Impurity B	0.003	$C_{36}H_{45}N_6O_8$	689.3299	2.9
Impurity C	<0.001	$C_{22}H_{31}N_4O_3$	399.2396	5.1
Impurity F	<0.001	$C_{22}H_{31}N_4O_2$	383.2447	0.4
Impurity H	0.013	$C_{25}H_{35}N_4O_4$	457.2815	1.5
Impurity I	0.002	$C_{22}H_{31}N_4O_2$	385.2603	0.5
Impurity J	0.012	$C_{22}H_{33}N_4O_2$	471.2607	0.3
Impurity M	<0.001	$C_{26}H_{39}N_4O_4$	471.2971	0.2
Impurity L	0.001	$C_{33}H_{52}N_6O_3$	290.2050	1.3
Impurity L		$2M+H^+$	579.4023	3.3
Impurity N	<0.001	$C_{22}H_{31}N_4O_4$	415.2345	4.3
Average error				1.0
Standard deviation				1.8

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