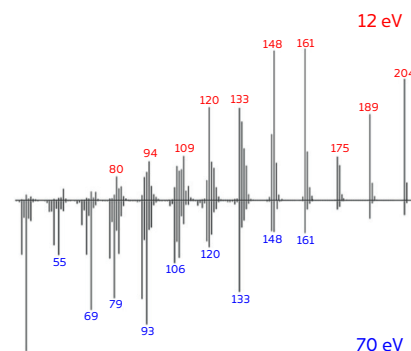


Technical note: Tandem Ionisation® – Revolutionary soft ionisation to enhance confidence in identification

This technical note describes the use of Tandem Ionisation for BenchTOF2™ mass spectrometers, to acquire both hard (70 eV) and soft (10–20 eV) electron ionisation (EI) in a single analysis. As well as describing the theoretical background to the technique and the benefits that stem from soft EI, we point out how Tandem Ionisation breaks new ground by providing sample characterisation in a single, streamlined workflow.



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Introduction

Electron ionisation (EI) is the production of ions by direct bombardment of analyte molecules by electrons. Conventionally, EI is used with an accelerating potential difference of 70 V, because at approximately 70 eV, the efficiency of energy transfer from electrons to most organic molecules is at a maximum and varies little with electron energy. This results in a relatively high degree of ionisation (and thus good sensitivity) and consistent mass spectra – the two principal reasons 70 eV EI is standard practice for GC–MS analysis. Indeed, some large commercial libraries, such as NIST and Wiley, have been developed almost exclusively on the basis of EI at 70 eV.

Nevertheless, such extensive fragmentation is not always desirable, and there are a number of gentler 'soft ionisation' techniques. These ultimately result in analyte molecules becoming ionised without giving them excess energy. The consequence of this is a limited degree of analyte fragmentation, meaning that a higher proportion of the original ionised analyte molecules reaches the detector intact. This ability to provide information about the unfragmented molecule makes soft ionisation of great value to analysts.

When a stronger signal from the molecular ion (and perhaps the larger fragments) is needed, it would seem logical to reduce the electron energy to just above the ionisation energy, i.e., 10–20 eV. However, EI suffers unacceptable losses in sensitivity when the ionisation energy is set below 20 eV. Therefore, GC–MS analysts have, by and large, accepted the limitations of EI at 70 eV, or resorted to other techniques to achieve soft ionisation, of which chemical ionisation (CI) is the most common.

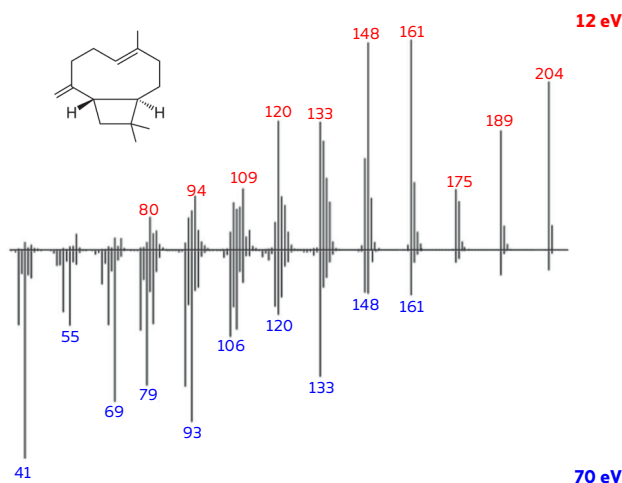
CI also results in a drop in sensitivity, but to a far lower extent than low-energy EI. Unfortunately, CI requires a different ion source configuration, additional source pressurisation and the use of reagent gases. If a single instrument is being used, this can be a time-consuming transition and a considerable drain on laboratory resources if required on a regular basis. Moreover, CI mass spectra are typically sparse, making library creation and searching impossible.

In this technical note, we describe Tandem Ionisation, an exclusive development in ion-source technology for BenchTOF time-of-flight mass spectrometers. We highlight how, by simultaneously providing reference-quality 70 eV spectra and complementary soft EI spectra, Tandem Ionisation has allowed analysts, for the first time, to fully benefit from the inherent advantages of soft ionisation for GC–MS, without any of its historic disadvantages.

What is Tandem Ionisation?

Tandem Ionisation provides fast switching (or multiplexing) between two ionisation energies in a single analytical run, enabling two sets of spectra to be acquired at the same time – reference-quality 70 eV spectra and 'soft' ionisation spectra with stronger molecular ions and less fragmentation, for improved discrimination between structurally similar isomers. This speed of multiplexing can be extended up to 200 Hz, enabling compatibility with GC×GC data acquisition.

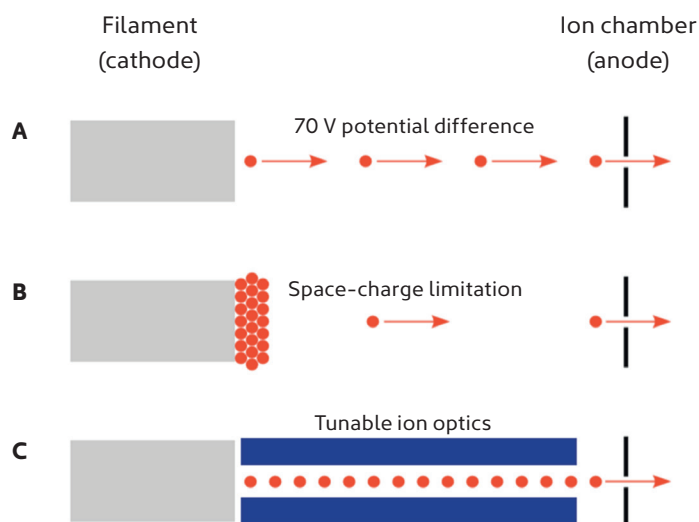
By enabling two sets of complementary spectra to be generated from a single run (as shown in Figure 1 for caryophyllene), Tandem Ionisation allows complex samples containing structurally similar compounds to be comprehensively characterised.

**Figure 1**

Comparison of 70 and 12 eV spectra for caryophyllene using Tandem Ionisation.

How does it work?

In a conventional EI ion source, there is a potential difference of 70 V between the positively charged ion chamber and the negatively charged filament ('electron gun'). This potential difference accelerates thermionic electrons away from the surface of the filament and through an aperture in the ion chamber wall (Figure 2A) for efficient ionisation of analytes eluting from the GC. In fact, the ionisation efficiency for most organic compounds is at a maximum at 70 eV, one of the key reasons for 70 eV to be chosen as the standard ionisation energy.

**Figure 2**

The operation of an electron ionisation ion source using (A) conventional (70 eV) ionisation energies, (B) low ionisation energies and (C) Tandem Ionisation.

However, a major problem stands in the way of 'soft EI' – loss of signal. This arises because achieving low electron energies with a conventional EI ion source requires a lower accelerating potential, which is insufficient to draw electrons into the ion chamber and results in clustering of electrons around the filament (known as the 'space-charge limitation', Figure 2B). This inefficient channelling of electrons to the ion chamber results in a small number of ions being generated, leading to an unacceptable loss of sensitivity.

To combat this, the Tandem Ionisation source for BenchTOF mass spectrometers uses a high potential difference to accelerate the electrons away from the filament, but then reduces their energy before they arrive in the ion chamber (Figure 2C). The ChromSpace® software controlling the BenchTOF allows the ionisation energy to be tuned for any two energies (between 10 and 70 eV) at a time. This feature is unique to BenchTOF mass spectrometers, due to their use of a pulsed electron beam, compared to other MS systems operating with continuous electron beams.

As for EI at 70 eV, soft EI is applicable to any analyte that can be vaporised. In addition – and unlike the majority of soft ionisation techniques – it does not require source switching, reagent gases or source pressurisation. Using this approach, the loss in sensitivity at low ionisation energy is minimised. In fact, in the following section, we will show how it can be partly compensated by a reduction in fragmentation and a reduction in background noise.

Reasons to consider Tandem Ionisation

Reduced spectral 'noise'

At lower energies, ionisation of carrier and background gases can be eliminated, resulting in much lower spectral 'noise'. In the example shown in Figure 3, the insets show the near-absence of $\text{CO}_2^{+\bullet}$ (m/z 44) from the 12 eV spectrum of *p*-cymene (the ionisation potential of CO_2 is 13.8 eV).

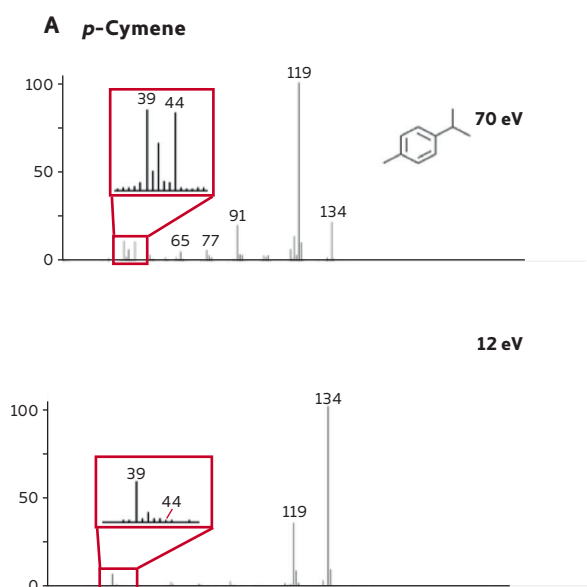
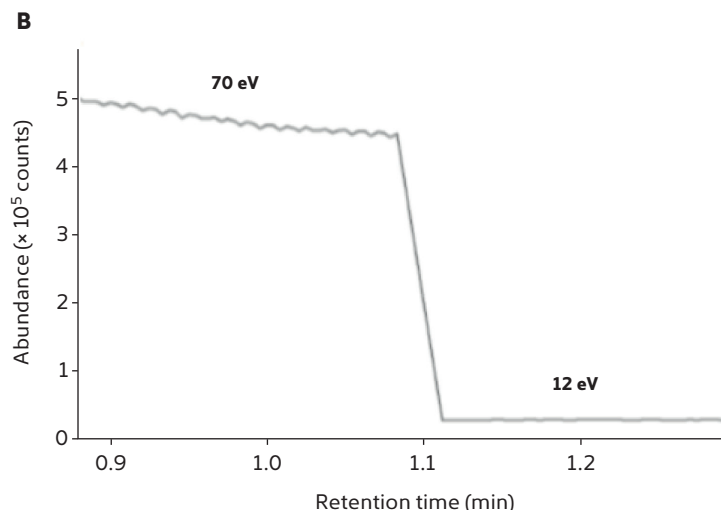


Figure 3

(A) Comparison of mass spectra, acquired using Tandem Ionisation, for *p*-cymene at 70 eV and 12 eV. The lower inset shows that 12 eV is insufficient to ionise the background gas CO_2 (MW 44). (B) The drop in chromatographic baseline when moving from 70 eV to 12 eV, due to the reduced ionisation of background/carrier gases at low eV (*continued on next page*).

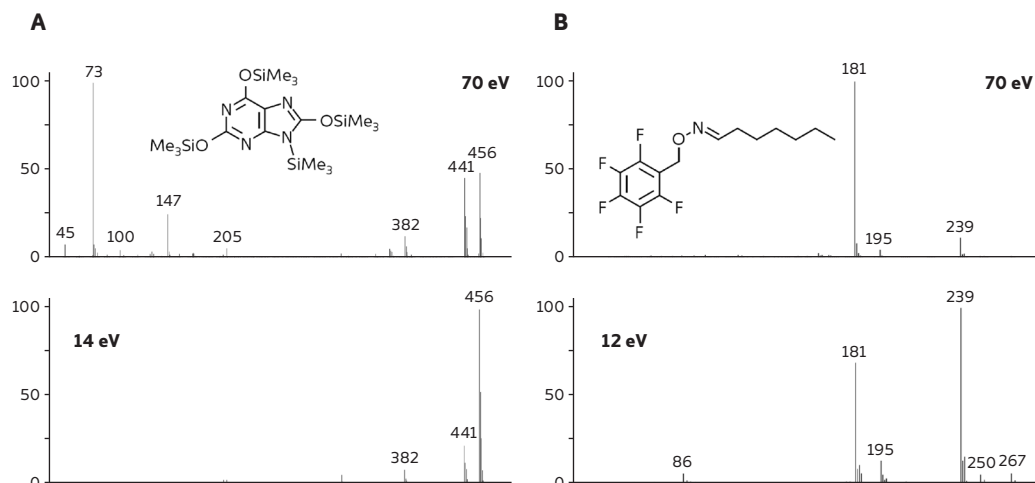
**Figure 3**

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Enhanced selectivity

The greatly reduced fragmentation of analytes (and background), along with the reduction in interference from ionised carrier gases, results in much cleaner spectra at lower energies.

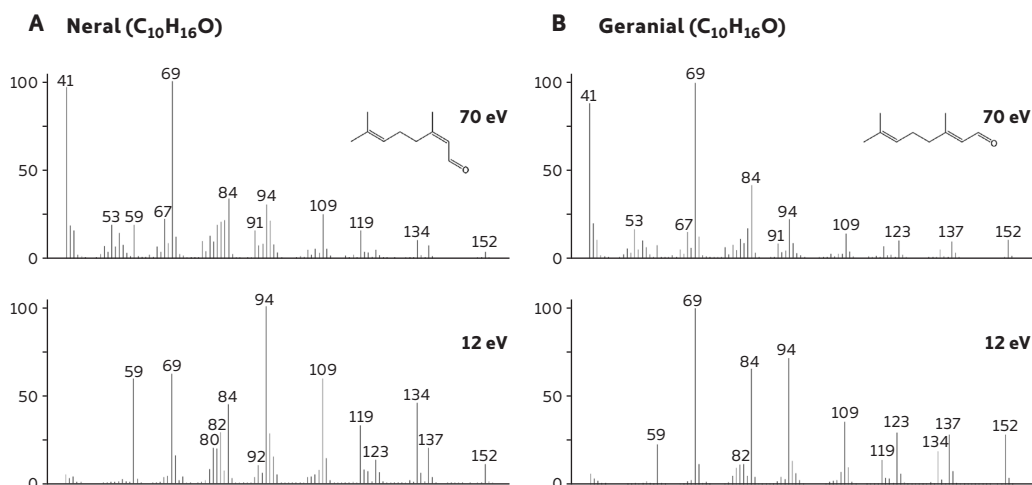
An excellent example of this enhanced selectivity is shown in Figure 4 for two derivatised compounds in urine. Tandem Ionisation has been shown to be highly effective at reducing the dominant ions associated with derivatisation^[1] for improved confidence in identification, especially within complex samples. In this case, Figure 4A shows a significant reduction in the intensity of the trimethylsilyl fragment (*m/z* 73) in the mass spectrum of tetrakis(trimethylsilyl)uric acid, while Figure 4B shows a reduction in the intensity of the 2,3,4,5,6-pentafluorobenzoyloxy fragment (*m/z* 181) in the mass spectrum of the corresponding heptanal oxime. In both cases, the reduction in the fragment ion response is accompanied by an increase in the signal from the molecular ion.

**Figure 4**

Comparison of 70 and 12/14 eV spectra for two derivatised compounds found in urine extracts, obtained by Tandem Ionisation.

Improved isomer speciation

In cases where analytes exhibit weak molecular ions and/or extensive fragmentation at 70 eV, analysts have previously resigned themselves to a low-confidence multi-analyte assignment ("This is probably A but could be B, C or D") – or indeed failure to identify the component at all. In such situations, Tandem Ionisation can be used to generate complementary low-energy spectra that aid correct and confident identification,^[2] as illustrated in Figure 5 for the isomers neral and geranial.

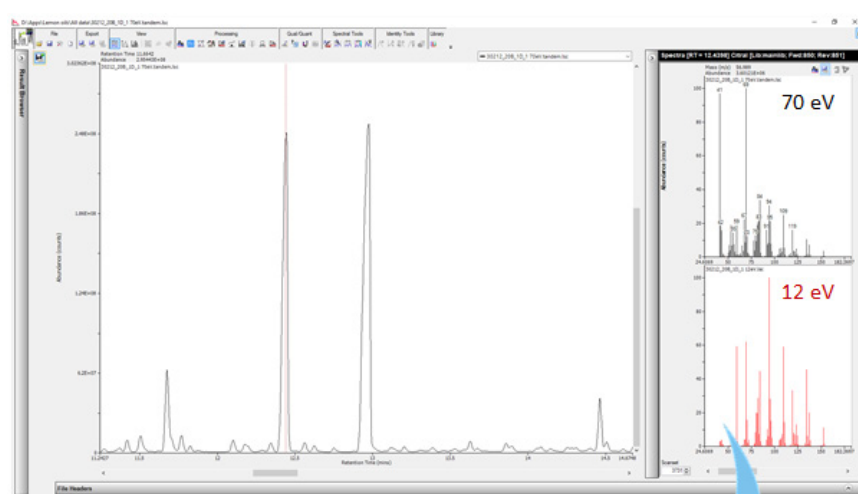
**Figure 5**

Comparison of the 70 eV and 12 eV spectra obtained for the isomers neral and geranial, showing the enhanced differences in ion ratios obtained when using soft EI, for improved isomer speciation.

Dual library searching

In contrast to other soft ionisation techniques, the consistent quality of spectra produced by BenchTOF2 is maintained at lower ionisation energies. This, combined with the degree of fragmentation that is retained at low eV, means that library searching of soft EI spectra is possible.

In ChromSpace software, streamlined tools are included to allow soft EI libraries to be used easily within streamlined workflows. The ability to interrogate the 'tandem' data in a single datafile means that dual library searching can be performed (Figure 6). Dual library searching adds confidence to identification by utilising both 70 eV and soft EI spectra to find the top library match across both data blocks in a single workflow. In this case, the 12 eV spectrum has the top library hit, confirming that the isomer is neral.



Search spectra against hard and soft EI libraries simultaneously

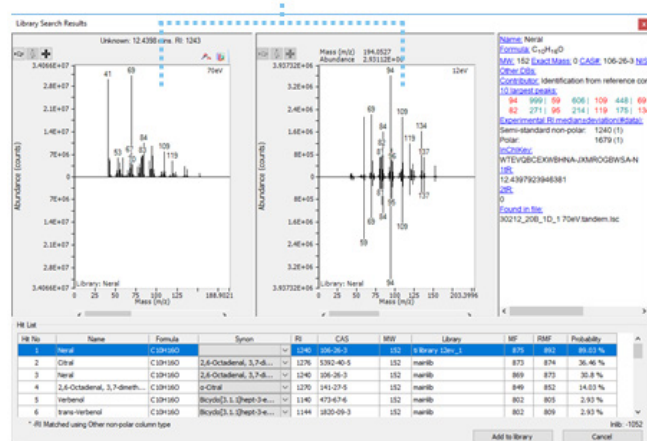


Figure 6

Using the tandem datafile, hard and soft EI spectra can be simultaneously searched against two sets of libraries to add confidence to identification.

ChromSpace software also includes time-saving tools to streamline the creation of such libraries. Once integrated, the spectra for all peaks in a chromatogram can quickly be added in a batch to populate hard and soft EI libraries simultaneously (Figure 7).

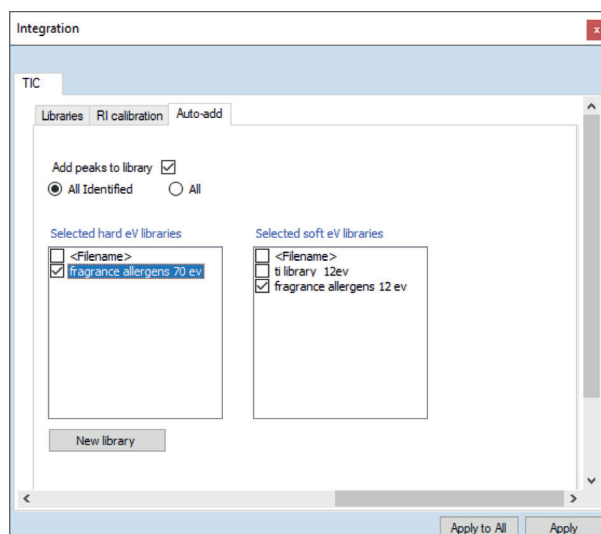


Figure 7

Simple tools to automatically add batches of spectra to soft EI libraries during integration.

Improved discovery workflows

The complementary nature of hard and soft EI spectra has been shown to improve chemical selectivity when identifying trace differences between complex mixtures, such as in petrochemical fingerprinting^[3] and in foodomics studies.^[4]

This enhanced selectivity can be harnessed in streamlined workflows within the ChromCompare+ chemometrics platform. ChromCompare+ uses an untargeted, tile-based approach to find the significant class-based differences between GC and GC×GC chromatograms.

During Feature Discovery in ChromCompare+, both sets of MS data (hard and soft EI) are utilised in a single workflow (Figure 8). The tandem data is used to confirm positive hits, thereby reducing the rate of false positives, minimising review time and increasing confidence in results.^[5]

Find out more about this approach in our technical note '[Improving discovery workflows using Tandem Ionisation data](#)'.

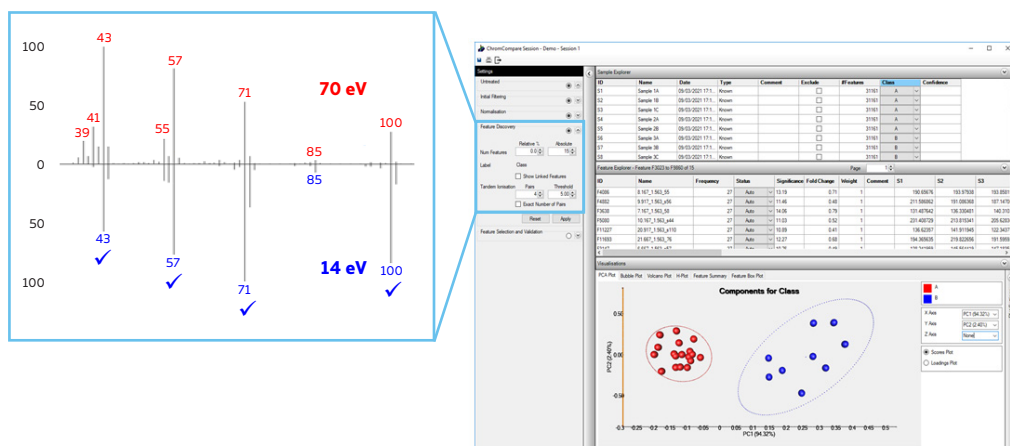


Figure 8

ChromCompare+ project window showing the Tandem Ionisation (TI) filter for Feature Discovery, as applied to the flavour profiles from two different quality grades of tea (labelled as class A and B).

Conclusions

This technical note has demonstrated that Tandem Ionisation for BenchTOF mass spectrometers provides:

- ▶ Patented technology for complementary hard and soft ionisation data in a single analysis.
- ▶ On-line soft ionisation with no hardware changes, reagent gases or user intervention required.
- ▶ Improved confidence in identification of isomers that are indistinguishable at 70 eV (such as terpenoids and hydrocarbons).
- ▶ ChromSpace software platform allows workflow to be fully automated and libraries of soft EI spectra to be created.
- ▶ Tandem data can be utilised in streamlined discovery workflows to confirm positive hits, thereby reducing the rate of false positives, minimising review and increasing confidence in results.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

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Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

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