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Short communication

Evaluation of a compact mass spectrometer for routine support of pharmaceutical chemistry

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ABSTRACT

The suitability of a recently introduced inexpensive, compact mass spectrometer detector is evaluated for supporting pharmaceutical chemistry investigations. While high performance/high cost MS detectors dominate the marketplace, there is growing recognition of the need for a small, inexpensive MS detector with reduced capabilities for supporting synthetic chemistry investigations, where reduced sensitivity and unit mass resolution are often suitable for solving routine problems. In this study, the fundamental performance characteristics of the recently introduced Advion compact mass spectrometer were evaluated, investigating the use of the instrument for routine product and impurity identification, reaction monitoring, evaluation of potential genotoxic impurities and study of high molecular weight biomolecules. In general, the results of the evaluation show this compact and inexpensive mass spectrometer to be well suited for providing reliable support for pharmaceutical chemistry investigations, with sub-nanogram limit of detection and impurity identification below 0.1% being possible in some instance.

1. Introduction

In recent years mass spectrometry (MS) data has become increasingly essential for carrying out drug discovery and development [1–3]. While many of the applications that rely on MS require high resolution/high cost instruments (*e.g.* proteomics, lipidomics, glycomics, *etc.*), MS support for routine synthetic chemistry applications is often quite straightforward by comparison, suggesting that MS instruments with reduced capabilities [4–6], could be sufficient fit-for-purpose tools for supporting pharmaceutical chemistry research. However, until very recently, major instrument vendors had concentrated on the development of high-end mass spectrometers, ignoring the need for smaller, less expensive, fit-for-purpose mass spectrometers with pared down capabilities that would be more suitable for supporting synthetic chemistry research.

Recently the results of a collaboration on the development of a miniaturized MS detector specifically targeted for supporting synthetic chemistry investigations has been reported, in which a compact (35 cm × 18 cm × 60 cm) chip-based, inexpensive (<$50,000) MS detector was shown to have acceptable performance for some synthetic chemistry workflows [7]. The miniaturized MS is capable of ionizing a range of compounds of general interest to process chemists. Although the sensitivity is somewhat reduced relative to conventional MS instruments, it is sufficient for some analysis to support synthetic chemistry, where sample is generally abundant and unit mass resolution is often sufficient. The detector sensitivity in the selected ion monitoring (SIM) mode was found fairly good, allowing trace-level detection of known components at the parts per million levels.

In this study the utility of the recently introduced Advion Compact MS for providing reliable support for pharmaceutical chemistry is investigated. The Advion system is a compact version of a conventional single quadrupole MS instrument. The system contains an external rough pump and like the chip-based MS system, is priced in the <$50,000 range, an important feature when considering a more widespread rollout to the large numbers of industrial synthetic chemists. While at 66 cm × 28 cm × 56 cm, the Advion instrument is smaller than conventional MS instruments, it is approximately twice as big as the chip-based system. In the article a series of studies have been conducted, including evaluation the performance characteristics of the Advion compact MS, comparing with the previously studied chip-based MS system and with conventional low-end MS instruments in current use for supporting synthetic chemistry applications, and various applications such as reaction monitoring, production identification, PGI evaluation, impurity identification, and largest molecules.

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2. Experimental

2.1. Instrumentation

The test system consists of an Agilent 1290 UHPLC system coupled to an Advion expression Compact MS detector. The Advion system (Advion Inc., Ithaca, NY, USA) was first introduced at 2012 Pittcon, and designed for in-hood use for synthetic chemists. The system contains an external rough pump, conventional ESI ion source, and single quadrupole MS detector in a vertical layout. The calibration vials, load/inject valve and API inlet are located on the front of the unit for easy access. The interface was coupled to a passive splitter delivering a flow of approximately 0.2 mL/min to the ion source (an approximately 3:1 split ratio). The comparison instruments were comprised of an HPLC system coupled to a conventional single quad mass spectrometer (Agilent LC/MSD, Agilent Technologies, Palo Alto, CA, USA) or a chip-based Microsaic MiD 3500 mass selective detector (Microsaic Systems Ltd., Woking GU21 5BX, UK). Comparison of specifications of three MS detectors were shown in Supplementary Materials Table S1. The Microsasic miniature MS still has the distinguished advantage in terms of footprint. All three instruments are not offering MS/MS capability for characterization of the analytes, and comparing to a conventional MS system, the major drawbacks of both compact/mini MS are lacking the ability to cycle through different acquisition modes within a single run, and have a smaller mass range.

2.2. Chemicals, reagents and HPLC method

All commercial chemicals used were of analytical grade. Acetonitrile (HPLC Grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate (NH_4HCO_2), formic acid, insulin chain B MS standard and angiotensin II MS standard were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q Gradient A10 from Millipore (Bedford, MA, USA). The HPLC method condition comprised a Waters BEH C18 (100 mm × 2.1 mm, 1.7 μm) (Waters Corp., Milford, MA, USA) column with mobile phase of A: 2 mM NH_4HCO_2 in H_2O (pH = 3.5, adjusted by formic acid) and B: 2 mM NH_4HCO_2 in 90% Acetonitrile and 10% H_2O (pH* = 3.5, adjusted by formic acid), and a gradient elution of 10–90% B in 8 min (1 min post; 40 °C; 1uL injection; 0.6 mL/min).

3. Results and discussion

The fundamental performance characteristics of the compact MS system were evaluated first. The sensitivity and linearity of the Compact MS was investigated using a typical active pharmaceutical ingredient (API) from Merck Research Laboratories in the positive ion mode using both full scan mode and selected ion monitoring (SIM) of the corresponding molecular ions (*m*/*z* 562), with tuning for the optimal signal intensity. The results (presented in Supplementary Materials, Fig. S1) show adequate performance of the compact MS for low level detection in both the scan mode and the SIM mode.

The linearity of detector response for the Compact MS was demonstrated using API concentrations from 0.5 μg/mL to 50 μg/mL, which represents a concentration range of importance for quantifying minor impurities. Fig. S1D shows the calibration curve for standard solutions of the test compound. The instrument offers two orders of magnitude linearity with an R-squared value of 0.9997 determined for the test analytes, comparable to a conventional system. It should be noted that LOD and linearity will be compound dependent, but our experience with the pharmaceutically relevant samples suggests that these range is fairly typical.

Next, the utility of the Compact MS system for confirming product identity, reaction monitoring and in-process analysis was investigated. While the confirmation of product identity can be relatively straightforward for high yielding reactions with few side products, MS detection shows its greatest value in troubleshooting problematic reactions. The example shown in Fig. 1 is a case in point. Attempted reduction of a nitrile to a primary amine afforded no peak at the anticipated mass of the desired product (*m*/*z* 213). The major peak in the reaction was identified as starting material (*m*/*z* 209), and MS revealed that the smaller, early eluting peak showed *m*/*z* 211, presumably coming from the incompletely reacted imine intermediate. These results quickly provided a window of understanding on an unsuccessful reaction,

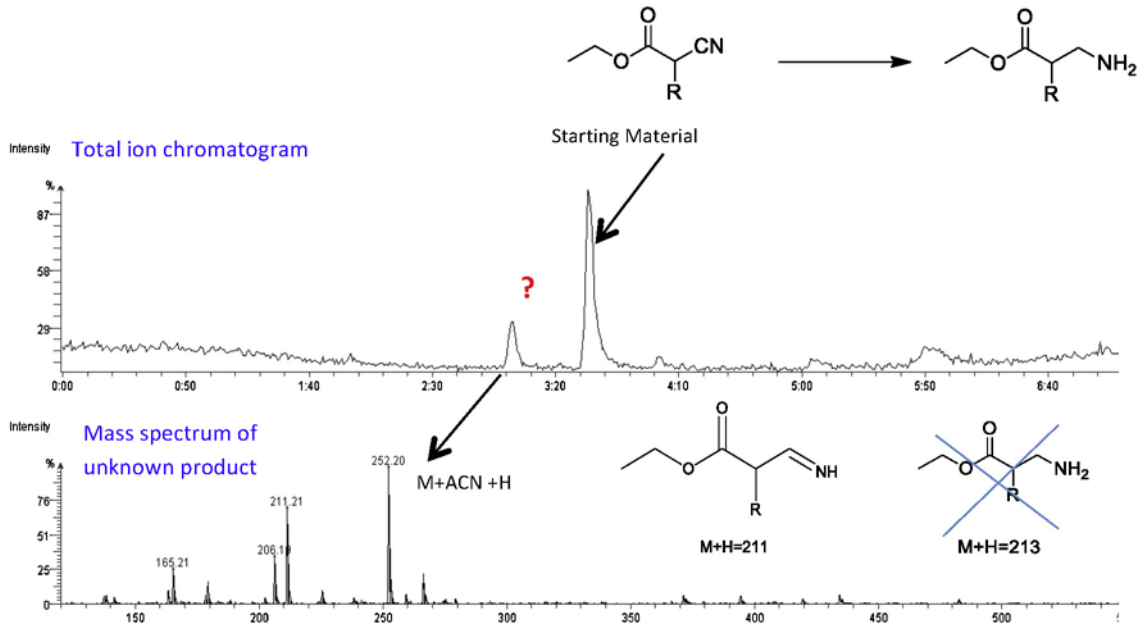

Fig. 1. Use of compact MS detector for synthetic reaction product confirmation. Top: total ion chromatogram; bottom: MS spectrum of minor peak; conditions: full scan positive mode; scan range 100–1000 *m*/*z*; scan time: 0.3 s; step: 0.2; capillary voltage: 350 V; capillary temp: 275 °C; source voltage: 25 V offset, 25 V gain.

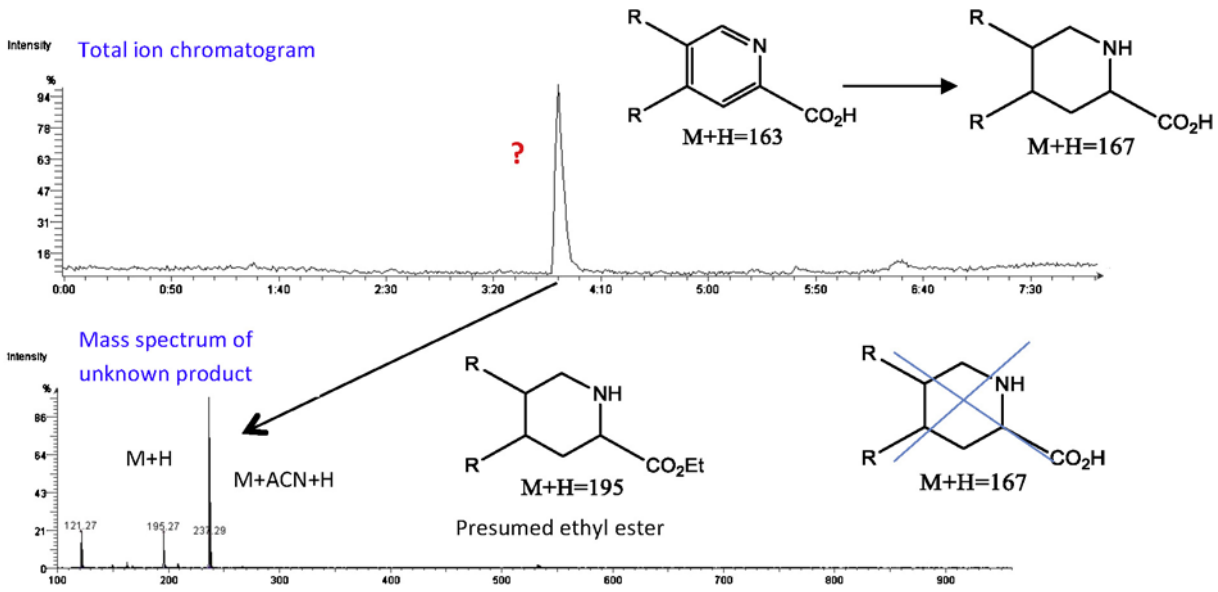

Fig. 2. Use of compact MS detector for synthetic reaction product confirmation. Top: total ion chromatogram; bottom: mass spectrum. Full scan positive mode; scan range 100–1000 *m*/*z*; scan time: 0.3 s; step: 0.2; capilllary voltage: 350 V; capillary temp: 275 °C; source voltage: 25 V offset, 25 V gain.

providing important information on what should be done to obtain the desired product.

Interestingly, in addition to the presence of the molecular ion at *m*/*z* 211, a significant peak at *m*/*z* 252 is observed as acetonitrile adduct [M+ACN+H]. The formation of solvent adducts during ionization process is not uncommon, but can create a significant impediment to rapid problem solving, as solvent adducts can often be confused with molecular ions and *vice versa*. Solvent adduct ions can sometimes be eliminated by adjusting instrument settings, but our initial investigations with the Compact MS have shown a marked propensity toward the formation of acetonitrile adducts that is difficult to suppress *via* instrument settings. Replacement of acetonitrile with other solvents such as methanol [8] is another possibility, although the almost universal use of acetonitrile as a preferred solvent for reversed phase HPLC suggests that this approach may be difficult.

The investigation of LC–MS analysis of an aromatic ring reduction by high pressure hydrogenation has been conducted. MS detection is a preferred tool for this type of reaction, as the final products often have very weak UV absorption due to chromophore

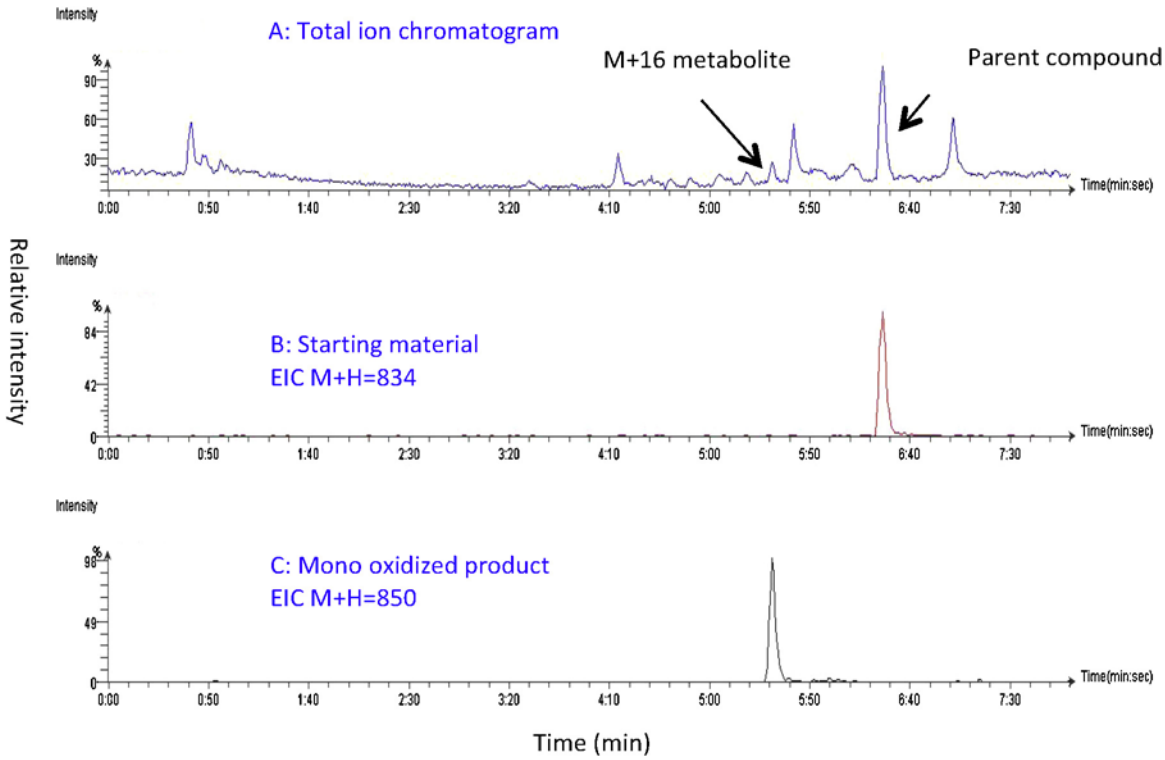

Fig. 3. Use of compact MS detector for identification of oxidation metabolite. (A) Total ion chromatogram; (B) extracted ion chromatogram of parent compound M+H = 834 with MS spectrum; (C) extracted ion chromatogram of M+16 metabolite, M+H = 850 with MS spectrum.

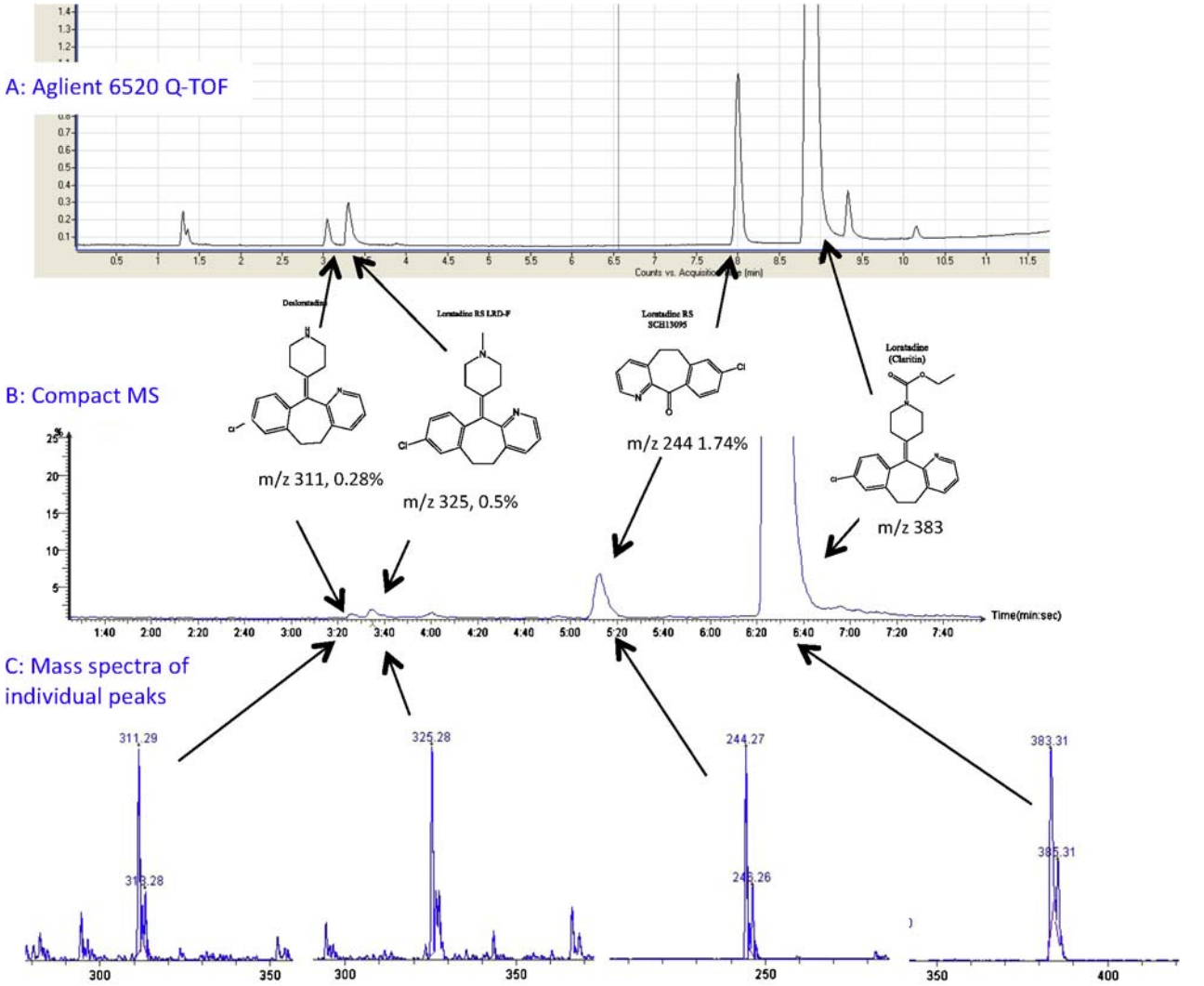

Fig. 4. Total ion chromatograms of loratadine drug substance standard spiked with different levels of known USP impurities (0.3% desloratadine, 0.5% loratadine RS LRD-F and 1.7% loratadine RS SCH3095). (A) Agilent 1290 LC-6520 Q-TOF. (B) Agilent 1290 LC-Advion compact MS. (C) Mass spectra of individual peaks.

destruction. Fig. 2 illustrates the use of the Compact MS detector in a typical end of reaction analysis, where the Total Ion Chromatogram (TIC) in full scan mode (100–1000 amu) clearly reveals the chromatographic peaks of interest. Examination of the mass spectrum of the major product from the reaction showed that rather than formation of the desired product, the corresponding ethyl ester was formed, presumably because of the use of EtOH as the reaction solvent. Notably, the acetonitrile adduct was once again observed as the major ion from this Compact MS system, whereas comparable studies using a conventional MS system showed only the protonated molecular ion.

Having demonstrated the suitability of the new instrument for providing MS support of many classical synthetic chemistry applications, study of some of the emerging areas of synthetic chemistry where low level impurity quantitation and identification is important, has been carried out. Synthetic chemists are increasingly becoming involved in the preparation and study of drug metabolites. When coupled with HPLC, MS has proven to be a preferred tool for such studies [9]. Fig. 3 shows an example of the use of the Compact MS to identify the only hit for substrate oxidation from a 96 well-plate screening of different oxidation conditions. The sensitivity of the Compact MS allows trace-level detection of the M+16 metabolite. Interestingly, the ACN adducts were not observed in this instance.

The identification of low level degradates and impurities is also important for stability studies or for assuring the quality of purchased raw materials and APIs [10]. The application of LC/MS not only allows the quantitation of such impurities, but also provides valuable clues to the identity of degradants and impurities, which can be crucial for addressing potential stability concerns. Fig. 4 shows the comparison of results from the analysis of a mixture of loratadine and known USP impurities using a high resolution Agilent 6520 Q-TOF instrument (Fig. 4A) and the Compact MS system (Fig. 4B). In this case, the full scan mode of the Compact MS instrument was able to detect and provide adequate sensitivity for low level (0.1%) impurities in the drug substance. In addition, mass spectra of the impurity peaks of interest clearly showed the nominal masses, along with halogen isotopic signatures, greatly facilitating impurity identification.

The use of the Compact MS system for studying a photodegradation problem is illustrated in Fig. 5. Upon artificial sunlight treatment, several impurities have been found to grow in a development compound sample using LC–MS with TIC detection. A mass spectrum of the major new peak formed upon sunlight exposure shows it to be the product of dearylation (loss of the methyl pyrimidine group), with both the [M+H] and the [M+ACN+H] peaks being observed in roughly equal abundance.

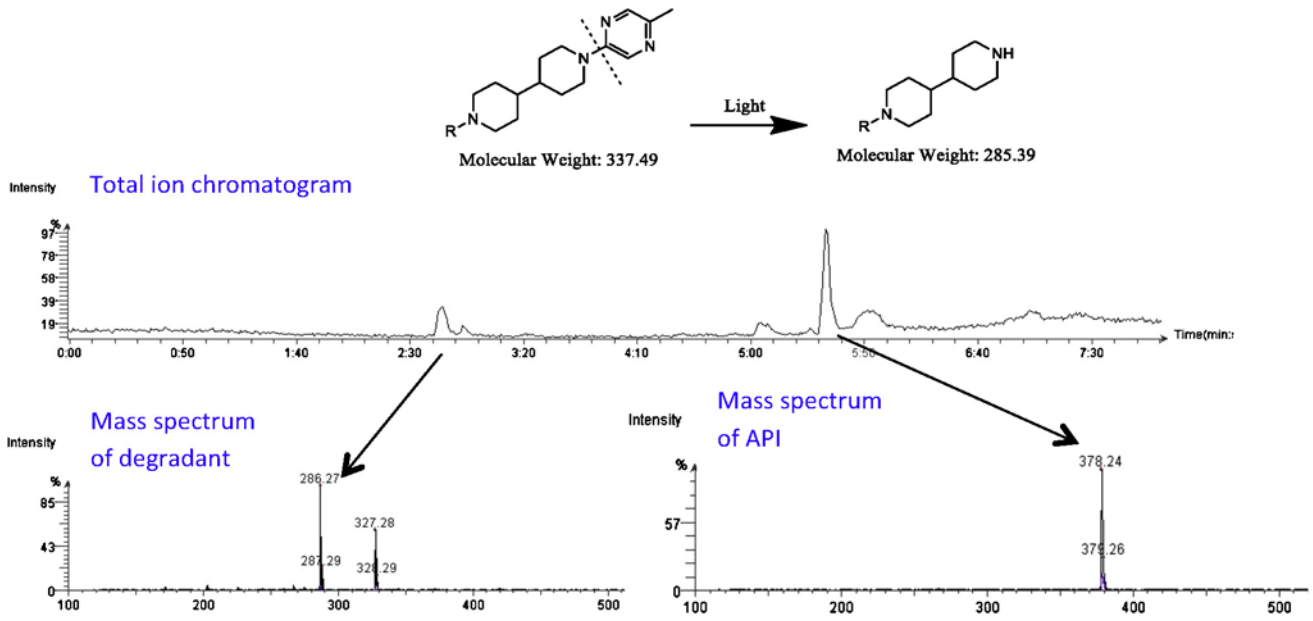

Fig. 5. Peak identification of API degradations. Top: total ion chromatogram; bottom: MS spectrum of peaks; conditions: full scan positive mode; scan range 100–1000 *m*/*z*; scan time: 0.3 s; step: 0.2; capillary voltage: 350 V; capillary temp. 275 °C; source voltage: 25 V offset, 25 V gain.

The presence of potential genotoxic impurities (PGIs) in drug substances has been a subject of considerable recent interest following the guidelines from regulatory agencies mandating the control of PGIs, sometimes at the parts per million (ppm) levels [11]. LC–MS has been shown to be a preferred tool for pharmaceutical chemists to investigate the presence and avoidance of PGIs [12].

Fig. 6 shows the use of the Compact MS to determine of the PGI residual level in API, the commonly used peptide coupling reagent, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) [13]. As EDC reacts readily with water, the development of an aqueous-based HPLC–MS method can be a challenge. An indirect approach in which all EDC present in the sample converted to the urea product,

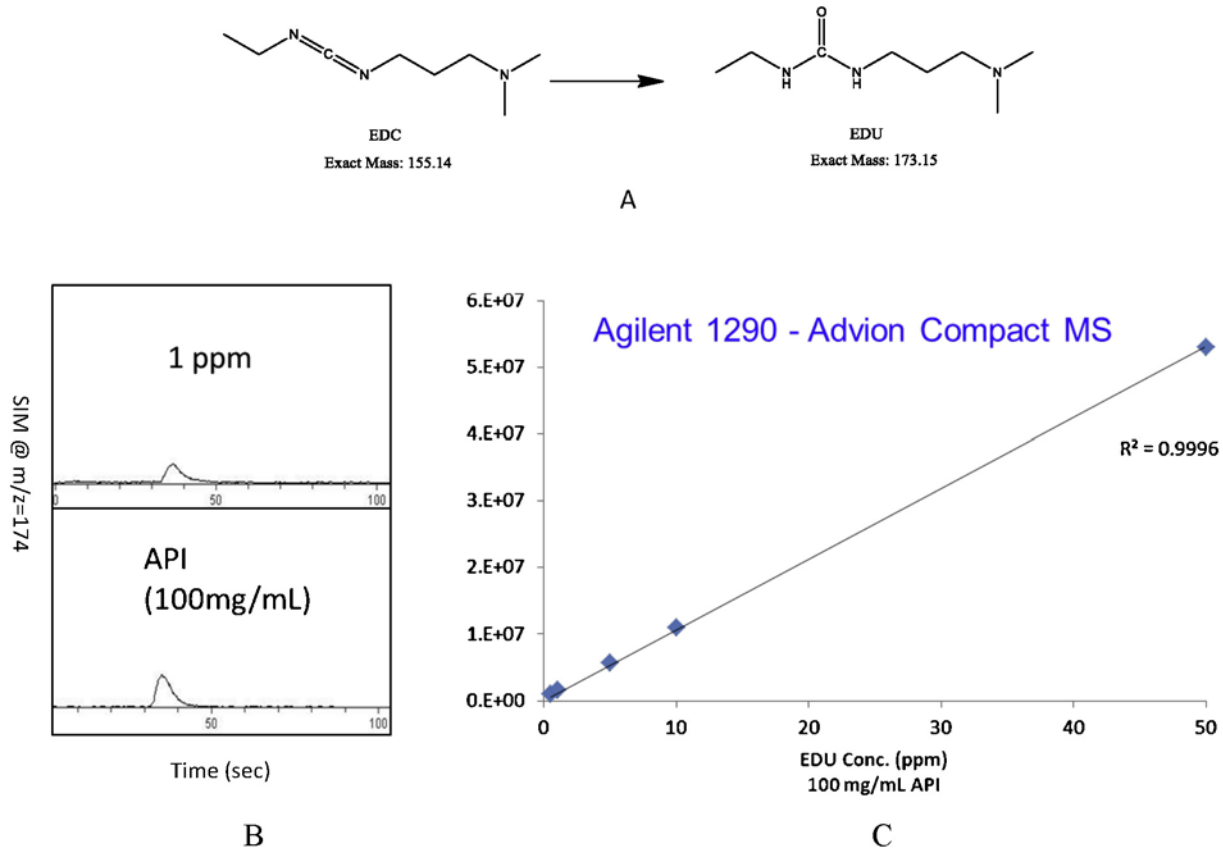

Fig. 6. Use of compact MS detector for detection of trace-level 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). (A) EDC hydrolyzed to EDU; (B) SIM chromatograms of 1 ppm standard and API sample; (C) linearity plots of 1–50 ppm EDU standards.

EDU ([M+H]: *m*/*z* 174), which can be conveniently monitored by LC–MS. It is important to note that this assay determines the total amount of EDC/EDU potentially present in the sample, and thus places an upper limit on EDC levels. Linearity and sensitivity were determined, and the method was used to evaluate an actual API sample. As shown in Fig. 6B, the SIM detection limit corresponds to an LOQ of 1 ppm, and when evaluating an actual API sample, a peak was detected at approximately 3 ppm, demonstrating a suitable method for determining residual EDC/EDU in drug substance.

In recent years, the boundaries between synthetic chemistry and biochemistry have continued to blur, with many traditional small molecule chemists increasingly becoming involved with the study and synthetic modification of peptides, proteins, oligosaccharides and oligonucleotides. Consequently, the ability of a MS instrument to support such studies is becoming increasingly important. Therefore, the application of small peptide (angiotensin II) and protein (insulin) analysis has been studied, and the results showed that this Compact MS detector is suitable for this kind of analysis (Supplementary Materials, Fig. S2). The mass range of 1200 allows the singly charged molecular ion of angiotensin II to be observed at *m*/*z* 1047, as well as the doubly charged [M+2H] ion at *m*/*z* 524. Similarly, the molecular ion for insulin chain B cannot be observed, however the multiply charged [M+Na+3H] ion is readily detected.

4. Conclusion

The Advion Compact MS detector is an important addition to the low cost, small MS instrument class. Studies of detection linearity, sensitivity in both full scan mode and selected ion monitoring (SIM) were found to be suitable for routine use in pharmaceutical chemistry problem solving, and often comparable to conventional MS instruments. Trace-level detection of known components in the SIM mode showed suitable performance for quantifications at the ppm level for PGI applications, and the full scan mode allowed convenient detection and adequate sensitivity for general problem solving for the identification of unknowns, although a propensity to form acetonitrile adducts can lead to some potential difficulties in interpreting mass spectra. Overall, the results of the evaluation show this compact and inexpensive mass spectrometer to be well suited for providing reliable support for pharmaceutical chemistry investigations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2014.01.029.

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Evaluation of a Compact Mass Spectrometer for Routine Support of Pharmaceutical Chemistry

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	Microsaic 3500 MiD	Advion expression Compact MS	Agilent 1100 LC/MSD G1946D
Ion Source	ESI	ESI with APCI option	ESI with APCI option
Ionization modes	Positive and Negative in sequential analyses	Positive and Negative in sequential analyses	Ability to cycle through four different acquisition modes on a scan-by-scan basis within a single run
Mass range	80 – 800 m/z	10 – 1200 m/z	2-3000 m/z
Mass accuracy	± 0.3 m/z	± 0.1 *m/z*	± 0.13 *m/z*
Mass resolution	0.7 FWHM	0.5-0.7 FWHM	0.13 FWHM
Sensitivity (reserpine, 10:1 S/N)	10 pg (SIM)	10 pg (SIM) 100 pg (Full scan)	1 pg (SIM) 50 pg (Full scan)
Flow rate range	< 1 uL/min (1: 3000-5000 spilt ratio)	10 µL/min to 500 µL/min	10 µL/min to 1000 µL/min
Scan modes	Full scan or 8 SIM channels	Full scan or SIM channels	Full scan and SIM
Scan rate	3,600 *m/z* units sec^{-1}	5,000 *m/z* units sec^{-1} (compatible with UPLC)	2500 u/s standard mode 5250 u/s fast scan mode
External floor pump	No	Yes	Yes
Dimension (H x W x D), Weight	35 x 18 x 60 cm, 27 kg	66 x 28 x56 cm, 32 kg	58 x 64 x 62 cm, 63 kg
Cost	~$50,000	~$50,000	~$100,000

Table S1 Comparison of specifications of Microsaic Mid 3500, Advion compact MS, and conventional MS (Agilent MSD)

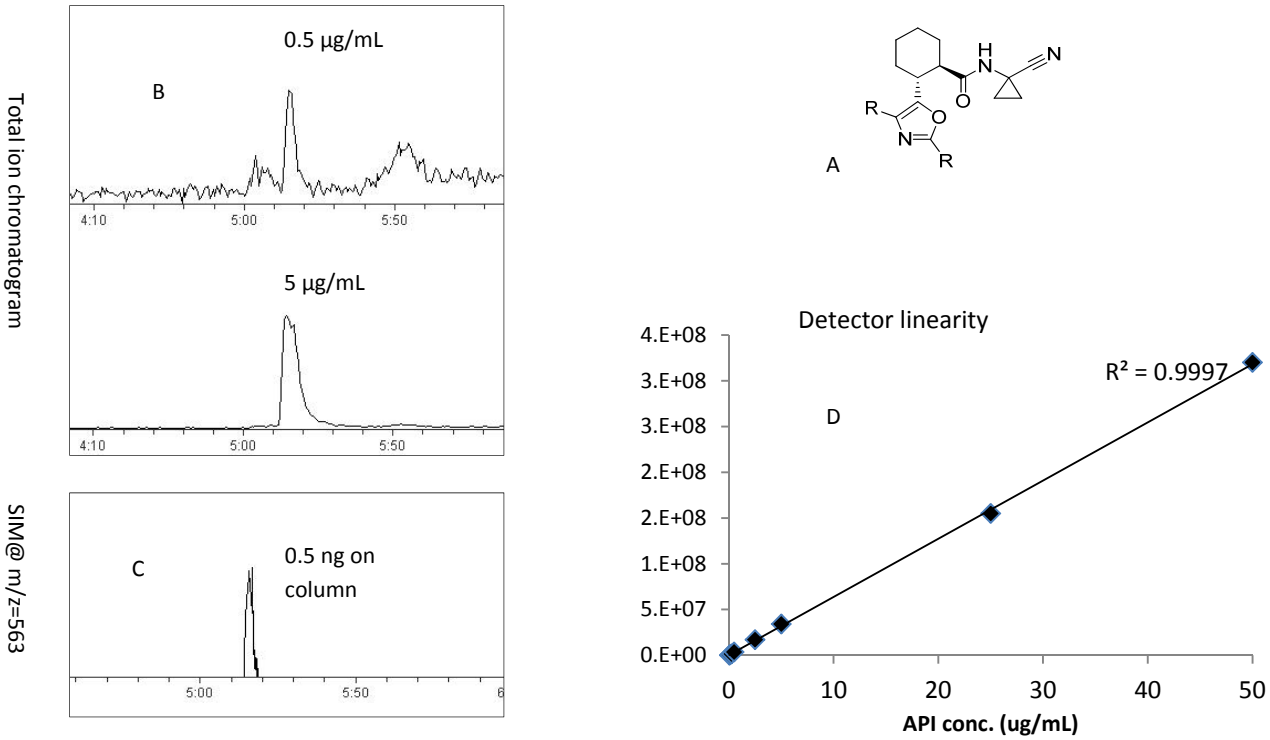

Figure S1: Limit of detection using the compact MS systems. The limits were determined both based on full scan mode and SIM mode at the appropriate molecular ion with 1 µL injected volume. A: Structure of the compound tested B:, Full scan positive mode; Scan range 100-1000m/z; Scan time: 0.5s; Step: 0.2; Capilllary Voltage: 350v; Capillary Temp: 275C; Source Voltage: 25v offset, 25v gain; C: SIM scan mode, M+H=563, 50 msec dwell time. D: Linearity plots for Advion compact MS (API concentrations: 0.5ug/mL to 50 ug/mL)

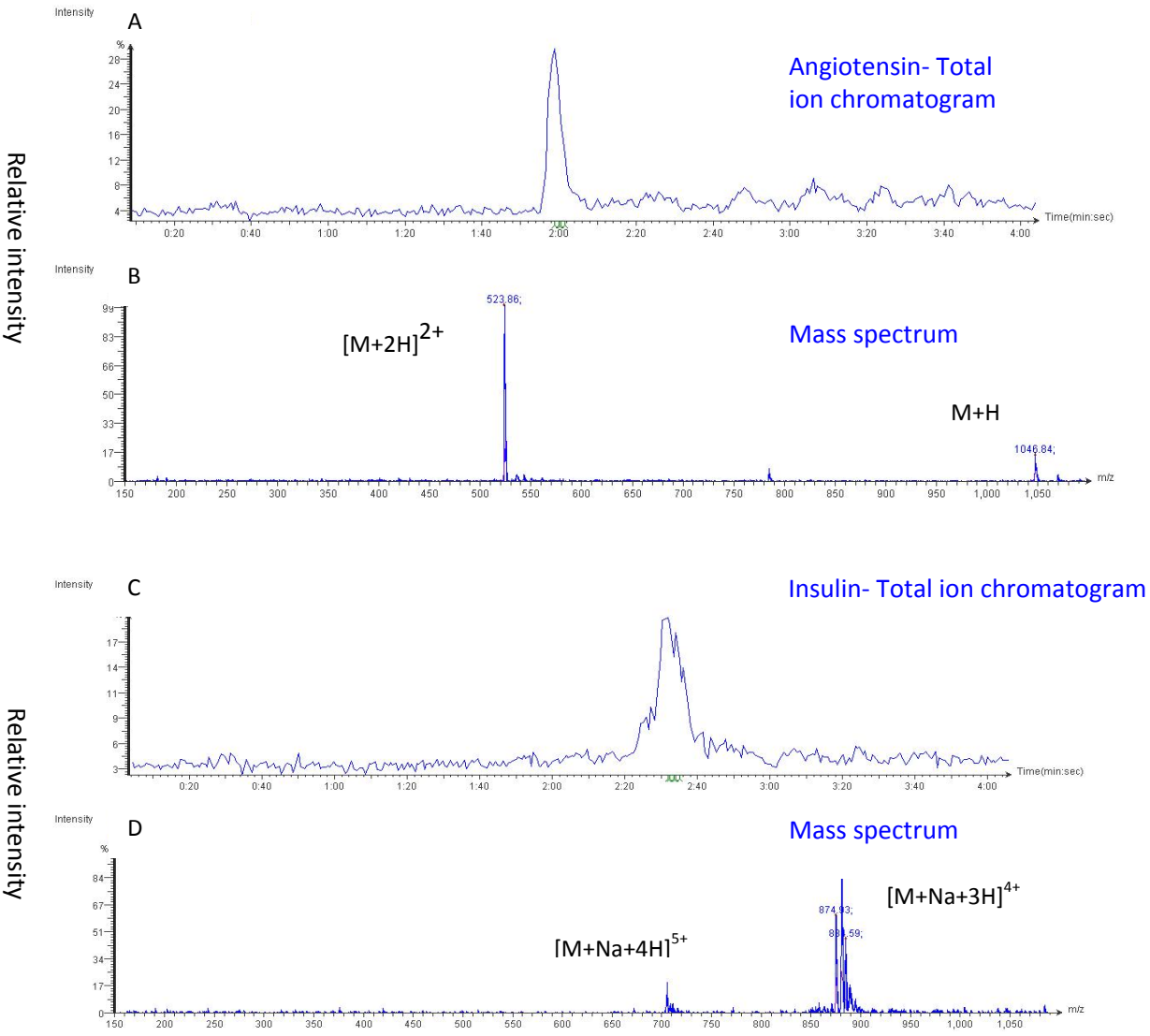

Figure S2: A: TIC of Angiotensin II MS standard (C50H71N13O12 MW: 1,046.18), 20 femtomole loaded on column ; B: MS of the peak; C: Insulin chain B Oxidized MS standard (C157H232N40O47S2 MW: 3495.89), 100 femtomole loaded on column ; D MS of the peak.