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# DART mass spectrometry: a fast screening of solid pharmaceuticals for the presence of an active ingredient, as an alternative for IR spectroscopy

With the advent of biosimilars and counterfeit pharmaceuticals the 'drug market' has been reported to contain a large number of products that do not contain any active ingredients.[1] Presently Infrared (IR) spectroscopy is used as the most common application for detecting active from inactive variants.

As a technique, IR spectroscopy has the user advantage of short analysis time, little or no sample preparation, and most poignantly, hand-held portability, enabling invaluable 'in the field' analysis. While sampling may be relatively easy, IR spectra of most pharmaceutical specimens contain a large number of complex bands that demand additional referencing (proprietary databases or spectrum libraries) in order for the investigator to make an accurate interpretation.

DART (Direct Analysis in Real Time) mass spectrometry was invented in 2005 by Cody and Laramee[2,3] during attempts to develop an atmospheric pressure version of the tunable electron monochromator for electron capture Liquid Chromatography/Mass Spectrometry (LC/MS),[4] and it brought forth a new methodology with the potential to provide an alternative investigative tool to IR spectroscopy for pharmaceutical quality-control purposes. Since its inception, DART has attracted interest from researchers due to the fact that it does not require sample preparation or chromatographic purification steps, making the technique extremely user friendly and simplistic.[3,5] DART mass spectrometry allows samples to be inserted directly into the ion source by forceps (if the object is solid) or by glass stick (for liquids) where it is evaporated and ionized; the resulting ions are detected by the mass spectrometer. The spectral results in DART mass spectrometry comprise signals corresponding to protonated molecules, $[M+H]^+$, of low-molecular compounds. This has the benefit of observing the spectra within a few seconds from the introduction of the sample. Such elements make DART mass spectrometry an extremely useful rapid identification technique for low molecular compounds and arguably an attractive alternative to the traditional IR approach in the analysis of pharmaceutical compounds.

Within general pharmaceutical analysis, the following DART routines have underscored the benefits of the technique: confirmation of the completion of reactions in synthesized pharmaceuticals,[6] analysis of mixtures separated on Thin Layer Chromatography (TLC) plates directly from their surfaces,[7] screening of drinks spiked with illicit compounds,[8] and quantitative analysis in both pharmacokinetic and metabolic analysis.[9] From these studies, utilizing DART technology to study counterfeit pharmaceuticals is a very encouraging prospect yet relatively little literature on this subject is available. So far, the recent studies have described the analysis of several solid pharmaceuticals and an ointment.[3,10–12]

**Figure 1.** Introducing the sample into the DART mass spectrometer.

Although small, this bibliography holds evidence for the further potential of DART in its application to pharmaceutical quality control.

In our own studies, a number of pharmaceutical tablets have been screened for the presence of active ingredients using DART mass spectrometry.[13–15] The unadulterated tablets were introduced into the air space between the DART ion source and the mass spectrometer inlet (Figure 1). The mass spectrum was rendered within seconds and with no requirement for spectral comparison and confirmation with proprietary libraries or databases, as with the IR technique. The complete procedure from sampling to data capture was infinitely more rapid.

In order to qualify the active ingredients within the batch of sample pharmaceuticals, mass-to-charge ratios ($m/z$) of the most intensive spectral signals were compared to the known molecular mass values of authentic compounds. The DART methodology positively confirmed the procedure for the detection of the active ingredients within the samples; Anapryline, Biseptol, Erespal, Glycine, Mexidol, and Nootropyl. By way of confirmation, the mass spectrum of Mexidol is presented in Figure 2. This pharmaceutical contained the active ingredient ethylhydroxypyridine (relative molecular mass M = 137 g/mol.).

As seen in Figure 2, the DART mass spectrum of Mexidol contains an intensive signal of protonated molecules $[M+H]^+$

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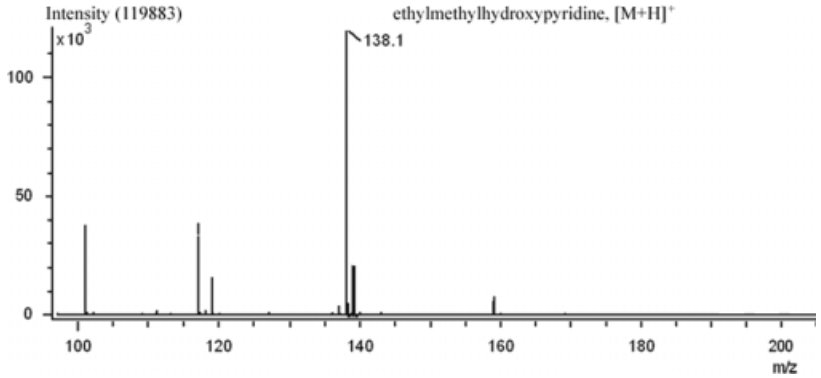


**Figure 2.** DART mass spectrum of Mexidol.

at *m/z* 138. Thus, the active ingredient in Mexidol can be positively and rapidly detected. The DART mass spectra of other analyzed pharmaceuticals were similarly simple to obtain and in all cases the presence or absence of an active ingredient was confirmed. In some cases the dimer ions $[2M+H]^+$ were observed, useful for additional confirmation of the active ingredients.

In these studies, the screening of pharmaceuticals for the presence of active and inactive ingredients with DART mass spectrometry shows great promise, in particular as an alternative labour-saving methodology to IR spectroscopy. It has the advantage of an extremely short spectral collection time, together with the provision of unambiguous results. The analysis of one sample takes only a few seconds and allows immediate mass spectral confirmation of the active or inactive ingredient. The DART system also encompasses online spectra interpretation during analysis, as such, the traditional comparison of recorded spectra with reference data, as with the IR approach, is no longer necessary. In fact, the molecular mass of the authentic active ingredient is the only salient information that is required for identification. In addition to these benefits, in confirmation of the ion compositions, elemental formulae can be estimated using the mass drift compensation function of the instrument software.

For all of its advantages, the application of DART in pharmaceutical quality control does not come without compromise. This may limit the future popularity of the current guise of the device. It is, after all, relatively expensive and can only be used within the laboratory setting, two distinct disadvantages from which the IR technique does not suffer.

When comparing DART mass spectrometry with the conventional mass spectrometric techniques, having an awareness of the following limitations is essential for the appropriate use of the instrument. First, the DART process is prone to forming erroneous ionization results such as $[M-H_2O+H]^+$ or $[M+NH_4]^+$ where $[M+H]^+$ could be the expected ions of the respective analytes.[16] This can result in complicating the spectral data.

DART does not distinguish between the isomers when using one ion $[M+H]^+$. Steiner *et al*. describe an attempt to induce the fragmentation by way of increasing the orifice voltage in order to distinguish the isomers by their different fragmentation patterns.[12] For some compounds it was shown that the differentiation between the isomers was possible with DART mass spectrometry however, the fragmentation patterns of some isomeric compounds did not yield any distinct differences. In such a setting it is advised that where reliable differentiation between the isomers is required, a chromatographic separation method should be considered.

The DART ion source was created originally to be coupled with the AccuTOF mass spectrometer (JEOL, Japan). As a result, most published research data has been obtained with the instrument in this configuration. Now that the instrument is capable of coupling with most commercially available mass spectrometers there is a greater potential to explore and expand the limits of the DART technology. DART experiments with analyzers other than TOF are described in a very few of the recent publications, and none of them are devoted to the analysis of pharmaceuticals.

In summary, DART technology shows considerable promise for rapid, 'investigator friendly' studies of active and inactive ingredients contained within pharmaceuticals, an issue that is extremely poignant with the current prevalence of biosimilars and counterfeit products. While DART technology is currently a relatively expensive *in situ* lab-based technique, its user-friendly approach renders it as an attractive, rapid, labour-saving tool in comparison to its traditional IR counterpart. As noted, the application of DART in this setting is not without its flaws but with additional investigative work and guiding literature to define its 'fit for purpose' parameters, its potential as a choice analytical tool for rapid analysis of counterfeit pharmaceuticals may be realized.

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