

## **Supporting Information**

### **Forensic Mass Spectrometry: Scientific and Legal Precedents**

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# History of Forensic Mass Spectrometry



## Introduction

Mass spectrometry (MS) has long held respect in the forensic community,<sup>1,3</sup> and contemporary legal critiques of forensic science agree that MS is “the near universal test for identifying unknown substances” and one of the gold standards of instrumental analysis.<sup>4</sup> Still, the MS community recognizes that even “gold standards” have their limitations and must be fit for purpose.<sup>5,8</sup> In the history of forensic MS, one of the first mass spectrometers in a forensic laboratory was in Birmingham, UK in 1973. Zoro and Hadley reported details of the workload of this first mass spectrometer in a fascinating summary in 1976 (see **Table 1**). The greatest proportion of cases involved

Number of cases	Type of case
59	Illegal possession of drugs
47	Suspicious death
18	Explosives
17	Arson
10	Miscellaneous
8	Administration of noxious substance
7	Driving under the influence of drugs
7	Malicious damage
4	Documents
2	Biology

the analysis of drugs, both in bulk form and in human bodily fluids. Fast forward 55 years to the modern era, and according to the Office of Justice Statistics, drug identifications remain the most frequently submitted evidence request in a typical forensic laboratory.<sup>9</sup>

**Table 1.** Distribution of case types of the first year of operation (1973) of a mass spectrometer in the Home Office Central Research Establishment in Birmingham, UK.<sup>9</sup>

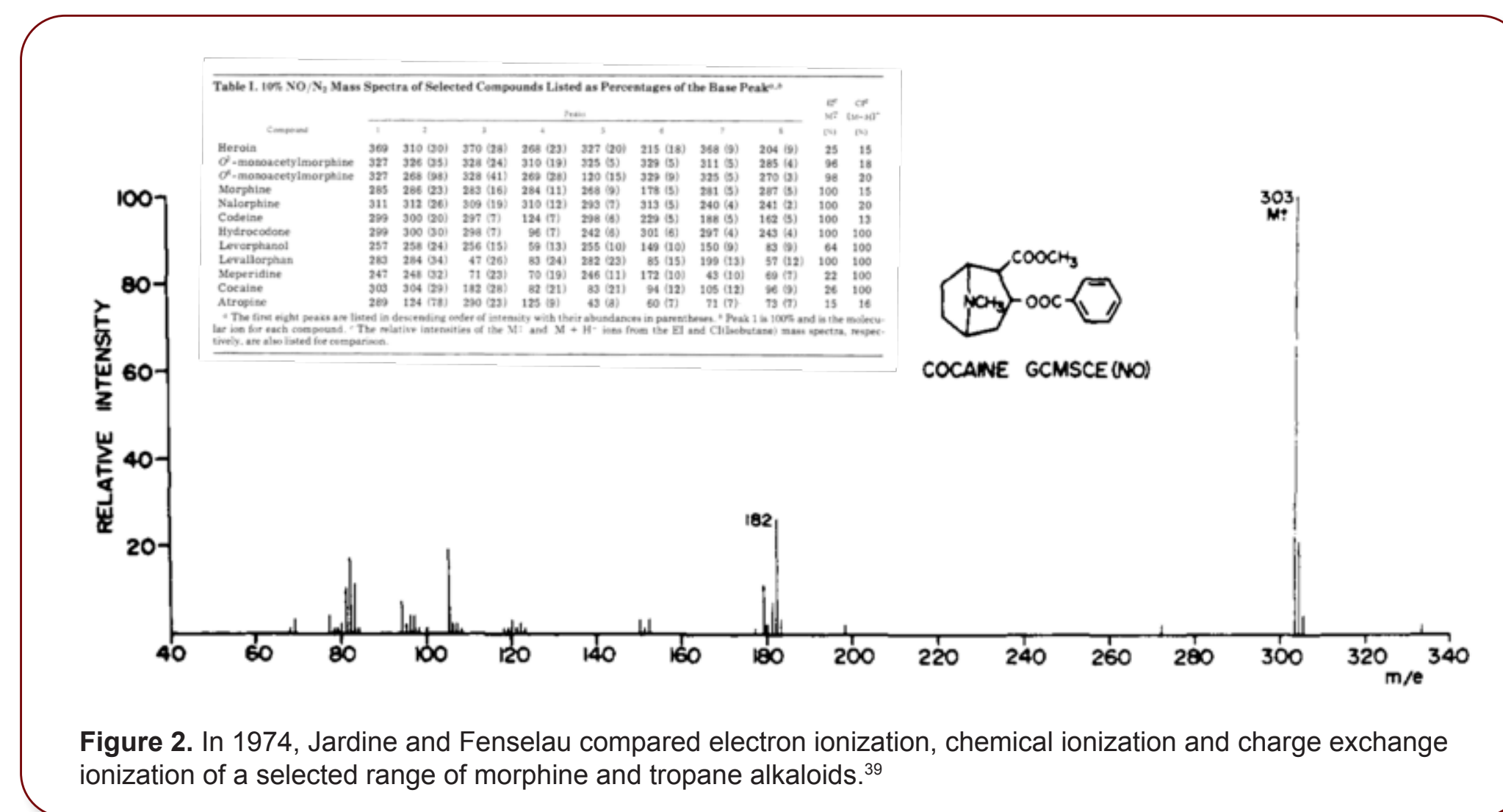
## Drugs and Toxicology

As described above, MS has found widest application in the analysis of drugs, drug metabolites and drug paraphernalia. In 1929, Bleakney developed the electron ionization (née impact) source for the analysis of gases and inorganic vapors.<sup>10</sup> He then extended the work to simple organics, like methane, in the late 1930s.<sup>11</sup> Although mass spectrometers were commercially available in the mid-1940s, they were big, expensive, oftentimes customized and difficult to operate.<sup>12</sup> Also, there was no guidance for interpreting the complicated spectra. Defying this latter problem, Beynon and McLafferty each contributed to the launch of organic MS in the mid-1950s.<sup>13-16</sup> From 1959 onwards, Biemann and Djerassi’s groups were prolific in extending MS’s capabilities to the analysis of natural products and botanical extracts, including various alkaloids, cannabis and cocaine.<sup>17-22</sup> At the Food and Drug Administration (FDA), in 1968, Martin and Alexander explained how they used high resolution MS (HRMS) and “cracking patterns” to help identify the hallucinogen dimethyltryptamine (DMT) in a casework sample.<sup>23</sup> They reported that “a problem that would have constituted a major research project a few years ago was reduced to an exercise problem in spectroscopic identification”.

In 1968, Coutts and Locock reported on the characterization of eight common barbiturates and their mixtures in pills and capsules.<sup>24</sup> Between 1968-1970, Bellman and coworkers at the FDA reported on the analysis of several hallucinogenic drugs using MS.<sup>25,26</sup> These early applications included lysergic acid diethylamide (LSD), mescaline, psilocin and psilocybin, among others. Others quickly followed suit.<sup>27,28</sup>

In 1970, Althaus et al. reported on the use of a gas chromatography-MS (GC-MS) system at Massachusetts Institute of Technology (MIT) to detect Darvon in stomach contents to solve a drug overdose case.<sup>29</sup> The case was apparently solved in about a day, which is a far cry from today’s backlogs but admittedly took a team of MIT MS experts to complete. By 1971, Fales’ group at the National Institutes of Health (NIH) had solved more than 100 overdose cases using GC-MS and computer-assisted database searching,<sup>30</sup> including the analyses of blood serum and stomach contents. In 1972, Skinner et al. reviewed the status of GC-MS for forensic toxicology.<sup>31</sup> In the same year, Green showed the potential of MS “fragmentography” to identify drugs from the headspace of drug samples, in pseudo real-time, with no sample preparation (besides dissolution in acid or base) (**Figure 1**).<sup>32</sup> He even showed the ability to detect alcohol in circulating blood *in vivo* (**Figure 1**)! Of course, the field of ambient sampling MS<sup>33</sup> has recently witnessed an enormous resurgence following the introduction of desorption electrospray ionization (DESI)<sup>34</sup> and direct analysis in real time (DART)<sup>35</sup> in the early 2000s.

In 1973, a Swedish team developed a GC-MS assay for  $\Delta^9$ -Tetrahydrocannabinol (THC) in human blood that was sensitive enough to detect if someone had smoked “one half-billionth of a gram”<sup>36</sup> The same year, Saferstein and Chao reported on the use of chemical ionization (CI)—which had been introduced by Munson



**Figure 2.** In 1974, Jardine and Fenselau compared electron ionization, chemical ionization and charge exchange ionization of a selected range of morphine and tropane alkaloids.<sup>39</sup>

and Field in 1966<sup>37</sup>—to analyze drugs and drug mixtures.<sup>38</sup> By 1974, Jardine and Fenselau added charge exchange ionization to the analysis of drugs (**Figure 2**),<sup>39</sup> and many other groups were adding to the “forensic mass spectrometry” literature. Many early forensic applications were captured in Fenselau’s comprehensive review of GC-MS in 1974.<sup>40</sup>

In 1977, MS data from the Environmental Protection Agency (EPA) was admitted as evidence in a case involving the detection of a pesticide known as tetrachlorodibenzo-*p*-dioxin (TCDD) in animal tissues from the Siuslaw National Forest.<sup>41</sup> The following year, a judge ruled to allow the MS test results as evidence in a capital murder case.<sup>42</sup> In 1978, GC-MS results were also admitted in a high priority case involving the American Meat Institute.<sup>43</sup> The concern was whether or not bacon had been adulterated by the addition of elevated levels of nitrosamines. MS was used in this case because it “is widely regarded as the best available technology”.<sup>43</sup>



**Designer Law for Designer Drugs:** In 1986, the Controlled Substance Analogue Enforcement Act (CSAEA, 21 U.S.C. § 813) was signed into law to control compounds that were “substantially similar” to drugs

that were scheduled in the Controlled Substances Act (CSA) of 1970 (Pub. L. No. 91-513; § 202, 84 Stat. 1236). In 1992, *United States v. Forbes* (806 F. Supp. 232), a judge ruled that CSAEA’s language was “unconstitutionally vague” and that alpha-ethyltryptamine (AET) was not substantially similar to the scheduled drugs dimethyltryptamine (DMT) or diethyltryptamine (DET). Instead of modifying the language, the DEA simply added AET to the list of scheduled drugs. In *US v. Washam* (2002, 312 F.3d 926), another judge found the language in CSAEA to be valid. Additional cases since 2003 have narrowed the language to include the “same core arrangement of atoms” test or the “structure and effect” test (e.g. *US v. Klecker*, 2003, 228 F. Supp. 2d 720).

In the 1970s, a ruling was overturned thanks to the ability of negative CI-MS to detect dibromopropanol—a metabolite of the flame retardant Tris(2,3-dibromopropyl) phosphate (Tris-BP)—in childrens’ urine.<sup>44</sup> In a review article on urinalysis in 1979, a probation officer named Bigger stated that although MS was “the most sensitive and specific technique available”, it was “too expensive and too slow to be commonplace”.<sup>46</sup> A survey of 100 crime labs in 1973 showed that mass spectrometers were the most desired piece of equipment, if unlimited funds were available,<sup>47</sup> and it only took a few years before GC-MS was commonplace in forensic laboratories.

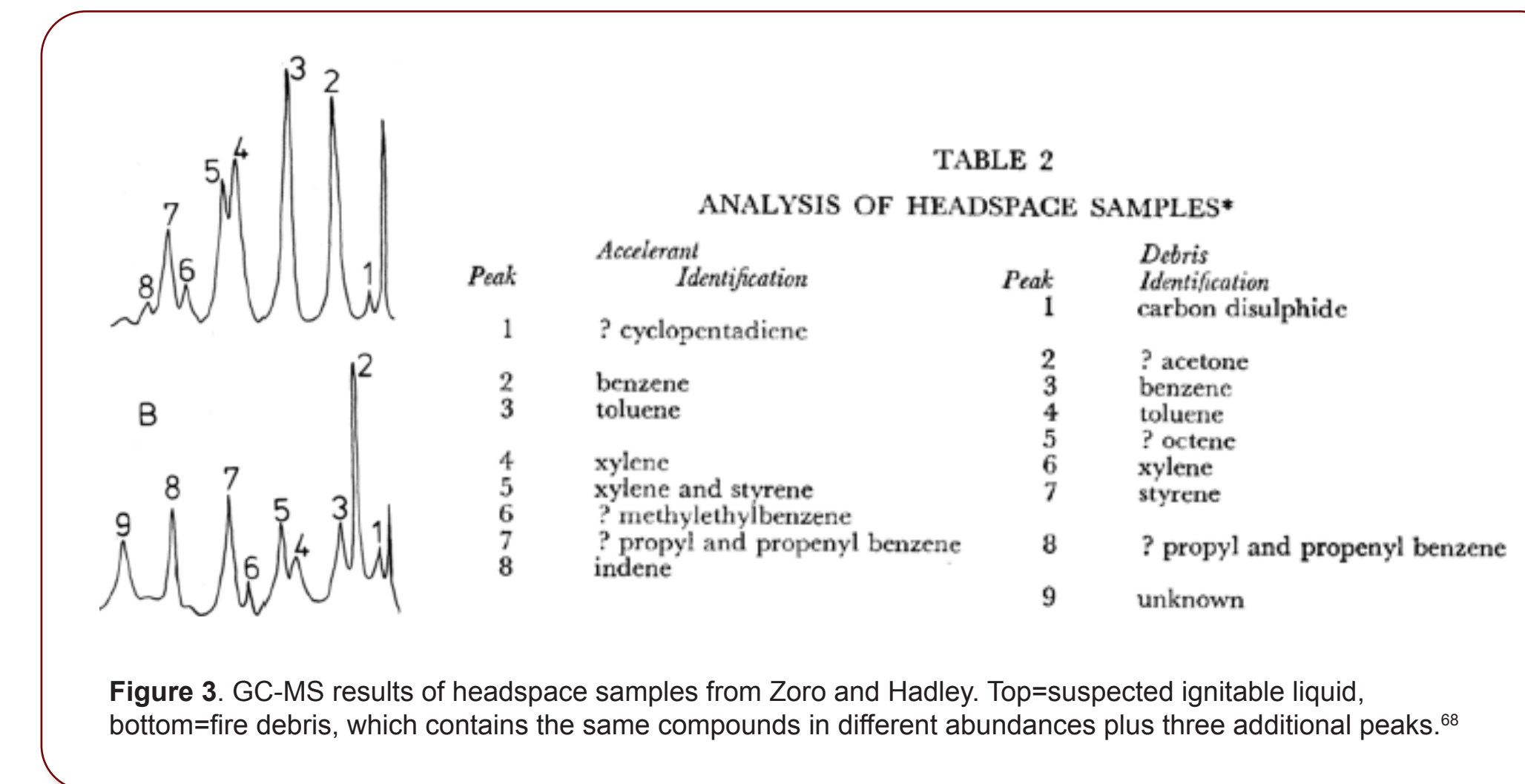
Although Djerassi and others had analyzed various tropane alkaloids using MS in the 1960s and early 1970s,<sup>18,48-50</sup> more than a dozen groups had contributed to the analysis of cocaine and its metabolites by the end of the 1970s.<sup>30,38,39,50-53</sup> However, Kondrat and Cooks were arguably the first to perform tandem mass analysis on cocaine in 1978.<sup>54</sup>

After the Controlled Substances Act of 1970, arguments continued over the need to distinguish harmless *d*-cocaine from the active drug, *l*-cocaine.<sup>55-58</sup> After some embarrassing problems with testimony about cocaine isomers,<sup>59</sup> one expert noted that *d*-cocaine “had also never been seen apart from *l*-cocaine”.<sup>60</sup> Thanks to his testimony, we no longer have to identify the isomers of cocaine!

At the 1972 Olympics in Munich, GC-MS screening produced 7 adverse findings from 2079 tests of athletes’ blood and urine.<sup>61,62</sup> The turnaround time averaged less than 24 hours per sample! Another major milestone was established in 1994 when Becchi et al. validated a method to distinguish exogenous from endogenous testosterone using GC-combustion-isotope ratio mass spectrometry (GC-IRMS).<sup>63</sup>

## Arson

In 1959, Joseph Nicol, a firearms technician at the Chicago police crime lab, suggested that crime labs at large universities or oil companies could run GC-MS tests for high priority arson cases.<sup>64</sup> In 1976, Zoro and Hadley<sup>65</sup> published a review article that outlined the use of GC-MS for ignitable liquids (née accelerants) in place of GC-flame ionization detection (GC-FID).<sup>66,67</sup> **Figure 3**, from Zoro and Hadley’s article, shows chromatograms of headspace samples of a suspected ignitable liquid and of the fire debris, the latter of which showed three additional compounds relative to the ignitable liquid.<sup>68</sup>



**Figure 3.** GC-MS results of headspace samples from Zoro and Hadley. Top=suspected ignitable liquid, bottom=fire debris, which contains the same compounds in different abundances plus three additional peaks.<sup>68</sup>

## Gunshot Residue (GSR) and Explosives

The composition of gunshot residue (GSR), also known as cartridge discharge residue<sup>69</sup> (CDR) or firearm discharge residue<sup>70</sup> (FDR), consists of the consumed and unconsumed particles from the primer and the propellant. The first tests used to determine whether or not someone had fired a gun by GSR was the paraffin test, which involved pouring hot paraffin wax—often painfully!—over a suspect’s hand and conducting a color test on the cooled wax.<sup>71</sup> Because of the general unreliability of the color tests,<sup>72-75</sup> alternative elemental and mass spectrometric approaches were developed, including neutron activation analysis (NAA)<sup>76</sup>, graphite furnace atomic absorption spectroscopy (GFAAS),<sup>76</sup> GC-MS,<sup>77</sup> inductively coupled plasma-MS (ICP-MS),<sup>78</sup> liquid chromatography-tandem mass spectrometry (LC-MS/MS),<sup>79</sup> and DESI MS/MS.<sup>80,81</sup> Whereas it was acceptable to use GC-MS in GSR cases in the 1980’s,<sup>82</sup> the American Society for Testing and Materials (ASTM E1588-17) developed a standard in 1994 that recommended scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM-EDS) to determine the presence of lead, antimony, and barium in the appropriate morphological particles; SEM-EDS remains the method of choice for GSR.<sup>83</sup>

As explained by Yinon in 1981—in his first of many books on the topic—the extreme sensitivity and selectivity offered by the mass spectrometer make it an ideal tool for the identification and forensic analysis of chemical explosives.<sup>84</sup> However, many explosives give no molecular ion by EI-MS, so much of the early work in the 1970s focused on chemical ionization.<sup>85-87</sup> Although mass spectrometers have been used as explosive detectors or “sniffers” since the 1970s,<sup>84</sup> they have only recently been identified by the National Research Council as desirable replacements for the considerably cheaper, but poorer resolution, ion mobility analyzers.<sup>88</sup>

A newer application to the field of forensics is the discrimination of explosives by using IRMS.<sup>89-95</sup> IRMS now meets Daubert criteria for admissibility,<sup>96</sup> and several reviews describe the huge variety of forensic applications of IRMS.<sup>97,98</sup>

### Comparative Bullet-Lead Analysis (CBLA)

The first successful attempt to match bullets with MS was in 1975 by Haney and Gallagher using spark source mass spectrometry (SSMS).<sup>99</sup> After the introduction of ICP-MS in 1980,<sup>100</sup> commercial instruments became a mainstay for trace metals analysis in just about every industry except the forensic community.

Because of problems with reporting the evidentiary value of CBLA, a court ruled CBLA by ICP-atomic emission spectroscopy (AES) to be inadmissible.<sup>101</sup> Although ICP-MS<sup>102</sup> and laser ablation (LA)-ICP-MS have the power to analyze sub-ppb level impurities, and even obtain isotopic analyses of trace elements, very few crime labs can afford the expense of these techniques.

## Trace, Fibers & Hair

Early applications of MS for hair analysis focused on the detection of trace-level inorganic impurities. Due to the low concentration of inorganic elements in human hair, only the most abundant elements could be studied. As early as the 1930s, scientists studied the concentration of iron in human hair using chemical extraction.<sup>103-114</sup> From the 1950s through the early 1960s, spectroscopic methods like flame atomic absorption (FAA) enabled the detection of the most abundant metals like iron and copper<sup>115-117</sup> and even mercury and lead exposure in cases of poisoning.<sup>118-120</sup> By the 1960s, NAA achieved new levels of detection for a few elements that were amenable to NAA.<sup>121</sup> In 1969, Yurachek *et al.* performed the first direct mass spectrometric study of trace elements in human hair using SSMS.<sup>120</sup>

Ion microprobe mass spectrometry (IMSS)—now secondary ion mass spectrometry (SIMS)—was first presented by Castaing and Slodzian 1962.<sup>122,123</sup> In its first forensic application, researchers at the McCrone Institute in 1977 used IMSS to link a strand of a suspect’s hair with hairs found at a firebombed Planned Parenthood clinic. After several appeals, IMSS was found to be reliable, but its application to human hair ultimately failed to meet the admissibility criteria of the day “because the analytical technique used had not attained general acceptance in the scientific community, nor were the experiments conducted shown to be sufficiently reliable and accurate.”<sup>124</sup>

In 1976, Zoro and Hadley described the application of pyrolysis mass spectrometry (Py-MS) to identify the presence of an antioxidant in the trace fragments of a polymer in a hack saw to those of the stolen polymer-coated cable.<sup>68</sup> Pyrolysis-GC-MS (Pyr-GC-MS) was introduced to the forensic community by Saferstein et al. and Hughes et al. in their 1977 studies on man-made fibers and polymers.<sup>125,126</sup> Since then, Pyr-GC-MS has also been applied to other trace polymeric materials, including binder, polymers and organic pigments in the automotive or architectural paints. In fact, such applications have been recommended by the support working group on materials analysis (SWGMAT) for more than 20 years.<sup>127</sup> Since the publication of these recommendations, methods such as LA-ICP-MS for trace metals in automotive paints<sup>128</sup> and IRMS for the analysis of white architectural paints have been applied with success, although not tested in court.<sup>97,129</sup> Pyr-GC-MS is still commonly used in today’s trace labs to study fibers and polymers.<sup>130</sup>

In 1978 Locke et al. demonstrated that SSMS could discriminate small glass samples,<sup>131</sup> and after Houck successfully coupled MS to ICP,<sup>132</sup> the forensic community slowly demonstrated the use of ICP-MS for the analysis of trace impurities in forensic glass samples.<sup>133-135</sup> By 2003, laser ablation (LA)-ICP-MS, which is now an ASTM standard,<sup>136</sup> was also demonstrated to be reliable for comparisons (trace) glass samples.<sup>137</sup>

## Conclusion

Mass spectrometry has a long and essential history in the legal community and continues to provide some of the most reliable evidence in the forensic sciences. Although MS techniques have evolved dramatically over the years, there remains the perpetual problem of adequately supplying the finances and education required for forensic laboratories to possess and use the latest capabilities. These issues were identified in the 1950’s<sup>64</sup> and 1970’s,<sup>138</sup> and they are equally relevant today.<sup>4</sup>

### Acronym Index

APCI	Atmospheric Pressure Chemical Ionization	IMMA	Ion Microprobe Mass Analyzer
CBLA	Comparative Bullet Lead Analysis	NIH	National Institutes of Health
CI-MS	Chemical Ionization-Mass Spectrometry	ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
CSA	Controlled Substances Act	IRMS	Isotope Ratio Mass Spectrometry
CSAEA	Controlled Substance Analogue Enforcement Act	LA-ICP-MS	Laser Ablation-ICP-MS
DART	Direct Analysis in Real Time	LSD	Lysergic Acid Diethylamide
DESI	Desorption Electrospray Ionization	NAA	Neutron Activation Analysis
EA-IRMS	Elemental Analyzer-Isotope Ratio Mass Spectrometry	PSAT	Pressure Sensitive Adhesive Tape
EI-MS	Electron Ionization-Mass Spectrometry	Py-GC-MS	Pyrolysis-GC-MS
EDS	Energy Dispersive X-Ray Spectrometry	Py-MS	Pyrolysis-Mass Spectrometry
EPA	Environmental Protection Agency	SSMS	Spark Source Mass Spectrometry
DMT	Dimethyltryptamine	SEM-EDS	Scanning Electron Microscopy/Energy Dispersive
FDA	Food and Drug Administration		X-ray Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry	TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
GC-MS/MS	Gas Chromatography-Tandem Mass Spectrometry	USDA	United States Department of Agriculture
GSR	Gunshot Residue		



# History of Forensic Mass Spectrometry

ASMS History Committee Poster, 2020

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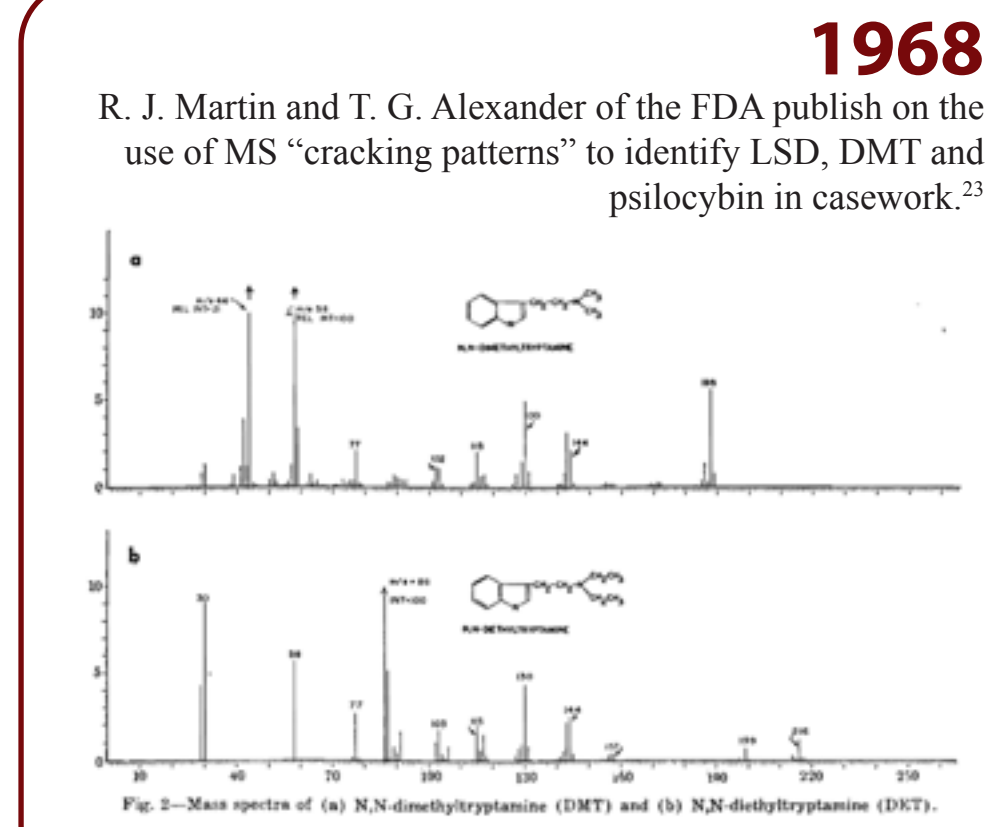


Pioneers of mass spectrometry, K. Biemann, C. Djerassi and F. L. McLafferty at the 38th ASMS Conference in 1990.

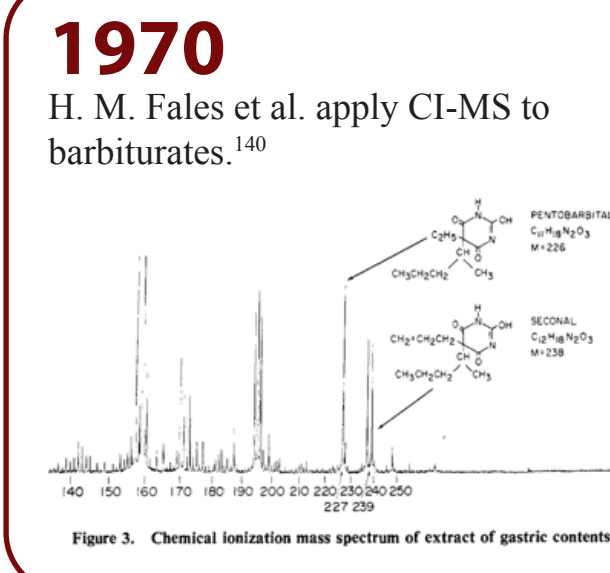


**1959**  
K. Biemann's use EI-MS to distinguish the stereochemistry of cyclic alcohols in natural products, including steroids.<sup>17</sup>

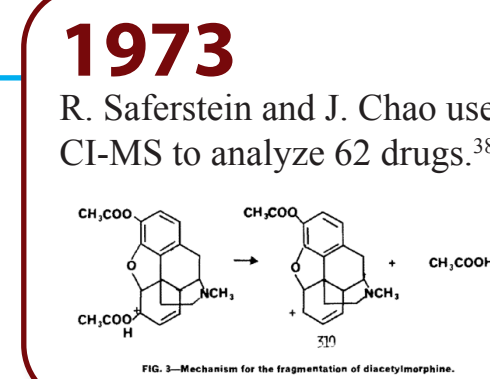
**1959**  
J. Nicol proposes the use of GC-MS for the analysis of ignitable liquid residues.<sup>64</sup>



**1967**  
After ~30 years of use, Cowan and Purdon conduct first comprehensive validation of the paraffin color test for gunshot residue (GSR); found the test to be unresponsive, but the test remains in practice for decades afterwards.<sup>75</sup>



**1970**  
K. Biemann's group publish a series of manuscripts using MS and computer-assisted searches to identify drugs and drug metabolites in overdose cases, including Darvon in a comatose patient.<sup>29</sup>



**1973**  
A survey of US crime labs showed that 2 out of 37 respondents had GC-MS capabilities.<sup>47</sup>

**1976**  
P. Bommer et al. first use isotope ratio mass spectrometry (IRMS) to distinguish between drugs with different origins; measured <sup>2</sup>H and <sup>13</sup>C ratios of different production batches of diazepam.<sup>142</sup>

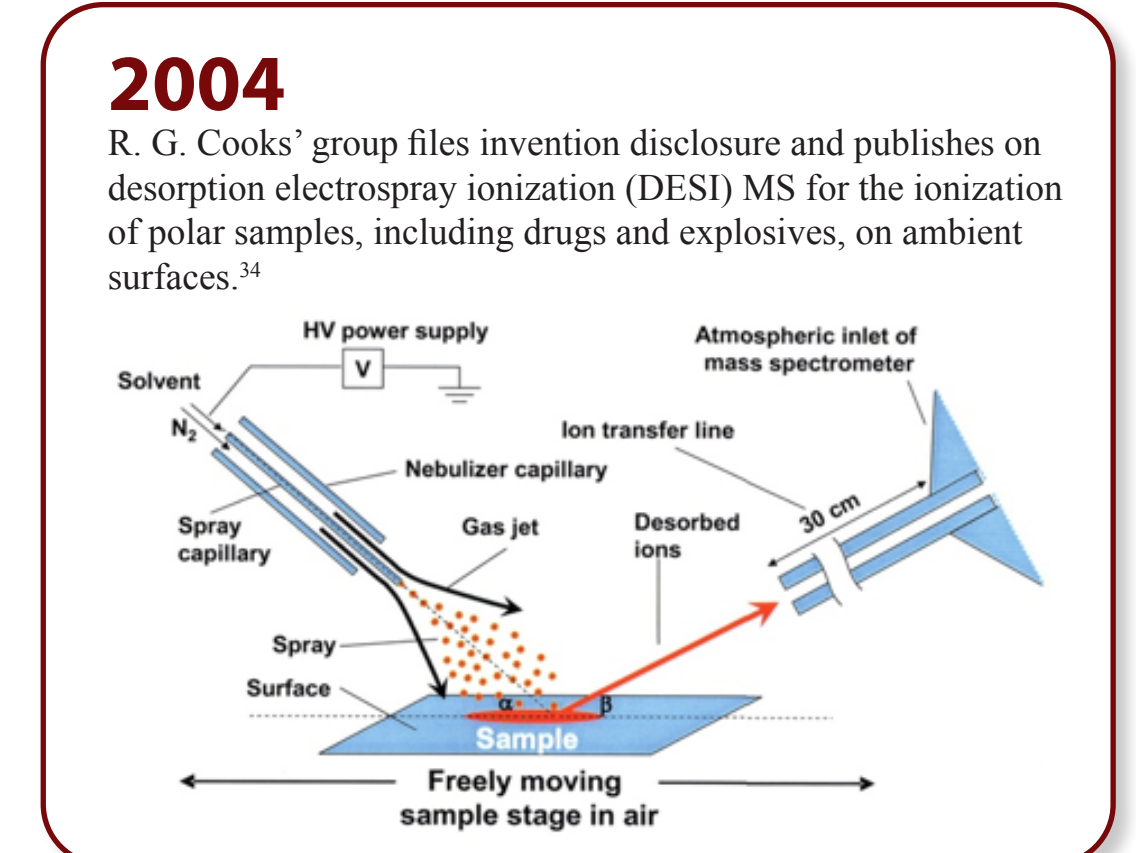
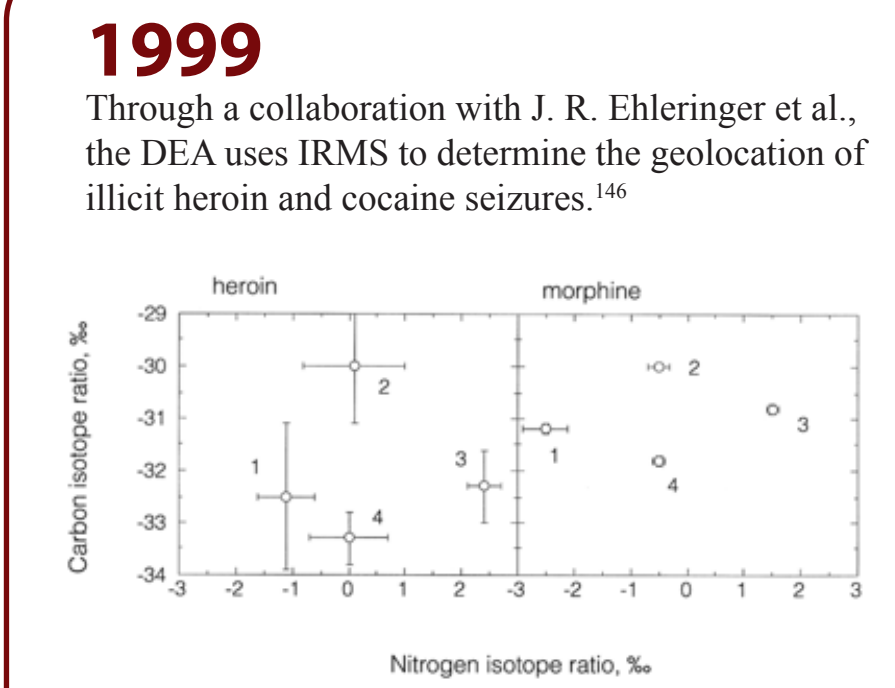
**1976**  
J. A. Zoro and K. Hadley publish a review of the use of MS in forensic laboratories in the UK; includes Pyrolysis-MS (Py-MS) of polymers and GC-MS of drugs and ignitable liquids.<sup>68</sup>

**1977**  
R. Saferstein et al. and J. C. Hughes et al. publish separate accounts of Py-GC-MS of man-made fibers and polymers.<sup>125, 126</sup>

**1977**  
In Citizens Against Toxic Sprays Inc. et al. vs USDA et al., the EPA uses low- and high-resolution MS to quantify the toxic herbicide TCDD in beef fat to levels of ~5 ppt.<sup>41</sup>

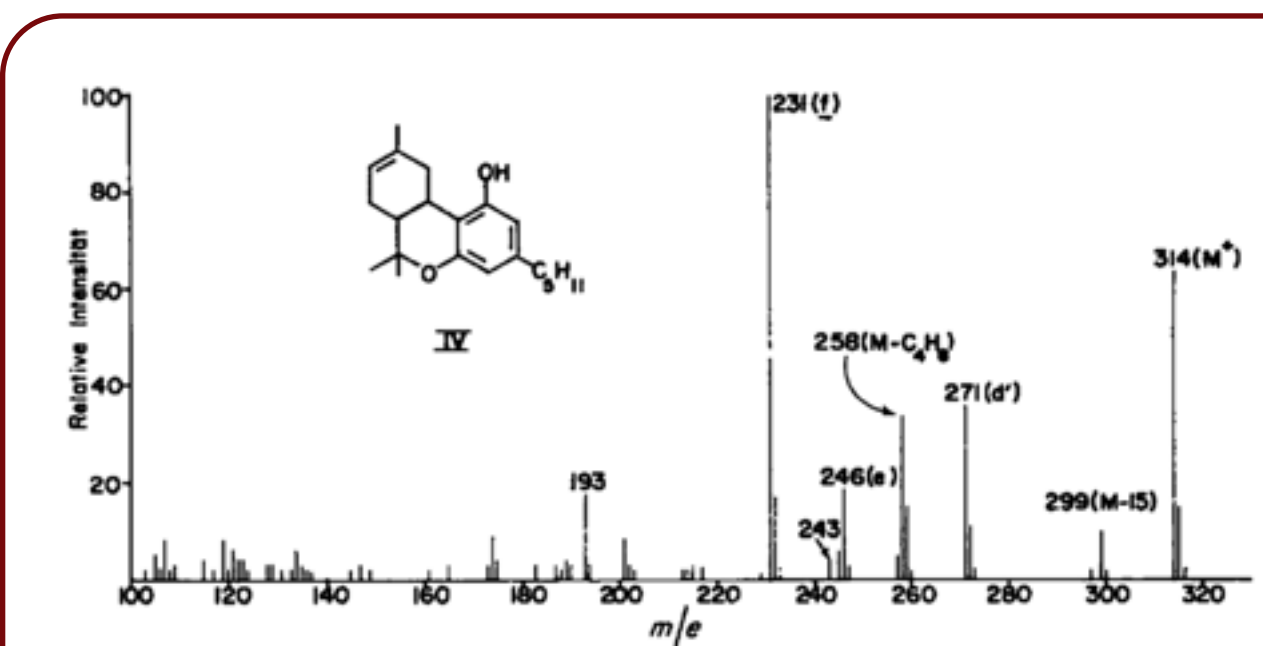
**1980**  
GC-MS used to disqualify a horse from a triecta race after detecting fentanyl in its urine.<sup>143</sup>

**1981**  
In US vs Posey, GC-MS evidence upheld for cocaine because "d-cocaine has never been seen apart from l-cocaine;" analysts no longer have to confirm the isomeric form of cocaine.<sup>60</sup>



**2008**  
W. Meier-Augenstein and I. Fraser use isotope ratio mass spectrometry (IRMS) to help identify the geographic origins and travel history of a mutilated murder victim.<sup>147</sup>

1950 1960 1970 1980 1990 2000 2010

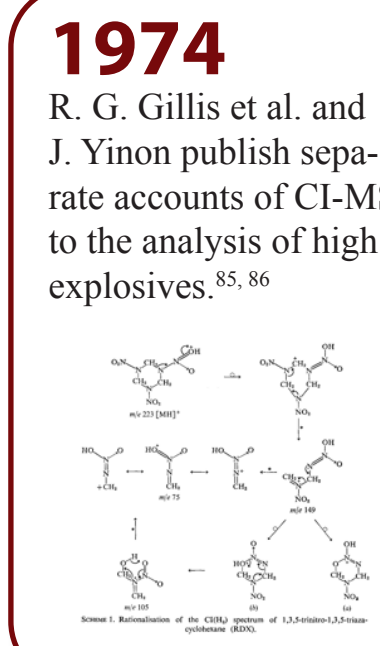


**1964**  
C. Djerassi's group publishes dozens of articles describing the mass spectral analysis of natural products, including tropane alkaloids and cannabinoids.<sup>18, 19</sup>

**1968**  
R. T. Coutts and R. A. Loeck analyze barbiturates from capsules and tablets using ether extractions and MS.<sup>24</sup>

**1971**  
The Federal Controlled Substance Act of 1970 becomes effective.<sup>141</sup>

**1971**  
G. W. A. Milnes' group at NIH report on the use MS and an automated search algorithm to solve hundreds of overdose cases.<sup>30</sup>



**1977**  
In *US vs Brown*, scientists from the McCrone Institute use an ion microprobe mass analyzer (AKA secondary ion mass spectrometry, SIMS) to compare the trace elements in human hair; technique found to be admissible after appeal, but the ultimate conclusions were not.<sup>124</sup>

**1978**  
To help enforce federal regulations, A. Blum et al. use GC-MS with a negative ion atmospheric pressure chemical ionization (APCI) source to identify metabolites of a flame retardant in the urine of children.<sup>44</sup>

**1978**  
R. W. Kondrat and R. G. Cooks introduce mass-analyzed ion kinetic energy spectrometer (MIKES) for the tandem MS of compounds from mixtures. Demonstrate the approach to identify drugs in various matrices, including cocaine from coca leaves.<sup>54</sup>

**1978**  
In *American Meat Institute vs Hon. Berglund* et al. GC-MS is used to confirm GC-thermionic emission analysis (TEA) results for nitrosamines in cooked bacon.<sup>45</sup>

**1978**  
J. Locke et al. demonstrate SSMS for classification of small glass fragments.<sup>131</sup>

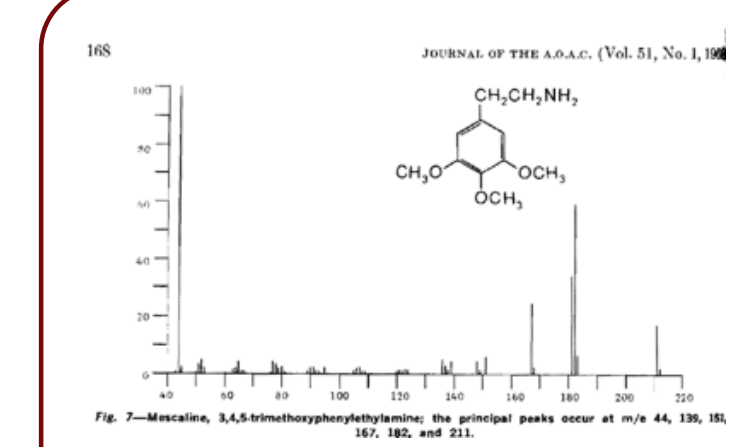
**1978**  
Judge finds MS evidence admissible for the identification of curare (of poison arrow fame) in several exhumed bodies.<sup>42</sup>

**1975**  
C. Fenselau and I. Jardine use charge exchange ionization to analyze various drugs.<sup>39</sup>

**1975**  
M. A. Haney and J. F. Gallagher use spark source-MS (SSMS) to differentiate bullets based on their elemental composition.<sup>99</sup>

**1972**  
In the summer Olympics in Munich, GC-MS produces 7 adverse findings in 2079 blood and urine samples. The turnaround time was <24 hrs for each sample.<sup>61, 62</sup>

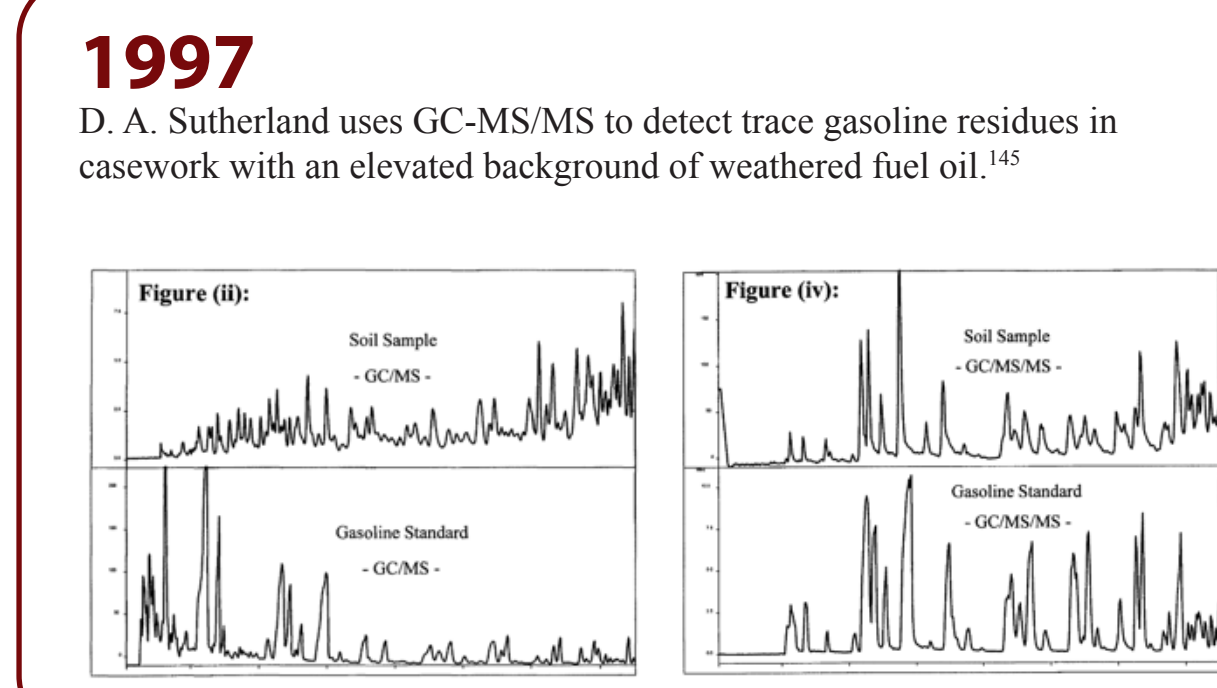
**1972**  
D. E. Green describes the real-time, in-vivo analysis of alcohol and drugs from humans using ambient sampling and "mass fragmentography."<sup>72</sup>



**1965**  
R. A. Brown publishes chapter on "The mass spectrometry of hydrocarbons," including common fragments and mechanisms for aromatics, alkanes, alkenes, cycloalkanes and polyaromatics; lays the groundwork for ignitable liquid residue determinations.<sup>139</sup>

**1990**  
A. Zuurhaar and L. Mullings apply inductively-coupled plasma-mass spectrometry (ICP-MS) to forensic analysis of glass samples.<sup>135</sup>

**1994**  
To combat doping in sports, Becchi et al. use GC-IRMS to distinguish exogenous and endogenous testosterone in human urine.<sup>63</sup>



**2003**  
R. B. Cody and J. A. Laramee file invention disclosure for direct analysis in real time (DART) for the direct analysis of non-polar molecules, including drugs and explosives, from ambient surfaces. Publish first results with H. D. Durst in 2005.<sup>35</sup>

Fig. 1. Schematic diagram of the DART-MS process showing gas in, needle electrode, gas heater, insulator cap, sample gap, grounded electrode, exit grid electrode, and MS inlet.



## References for: History of Forensic Mass Spectrometry ASMS History Committee Poster, 2020

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