A preliminary study of the analysis of *Cannabis* by supercritical fluid chromatography with atmospheric pressure chemical ionisation mass spectroscopic detection

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Science & Justice 1997; 37: 91–97

Received 31 May 1996; accepted 27 September 1996

A rapid method is described for the analysis of *Cannabis* products by supercritical fluid chromatography (SFC) coupled to atmospheric pressure chemical ionization-mass spectroscopic (APCI-MS) detection. The method had a shorter analysis time than GC-MS methods, without the need for derivatization prior to analysis. It was also faster than HPLC methods, with better resolution and definitive identification. Linearity of detector response to cannabidiol, Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol and cannabinol was established, the detection limits for mass on column being 0.55 ng, 1.20 ng, 0.69 ng and 2.10 ng respectively. The technique offers a means by which *Cannabis* products can be definitively identified in a single chromatographic run. Application to casework samples is described.

Une méthode rapide d'analyse des produits du Cannabis par chromatographie supercritique fluide couplée à une détection par spectroscopie de masse de ionisation chimique à pression atmosphérique est décrite. La méthode est plus rapide que les méthodes de GC-MS sans les besoins de dérivatisation. Elle est également plus rapide que les méthodes HPLC, avec une meilleure résolution et une identification définitive. La réponse du détecteur au cannabidiol, au Δ8-tétrahydrocannabinol, au Δ9-tétrahydrocannabinol et au cannabinol était linéaire et les limites de détections pour la masse en colonne était de 0.55 ng, 1.20 ng, 0.69 ng et 2.10 ng respectivement. La technique offre un moyen qui permet d'identifier définitivement les produits du Cannabis en une seule analyse chromatographique. L'application à des échantillons réels est décrite.

Es wird eine schnelle Methode zur Analyse von Cannabis Produkten mittels superkritischer Flüssigkeits-chromatographie gekoppelt mit der chemischen Ionisationsmassenspektroskopie bei atmosphärischem Druck (APCI-MS) beschrieben. Mit der Methode werden gegenüber der GC-MS die Analysezeiten verkürzt, ohne daß die Proben vorher derivatisiert werden müssen. Das Verfahren ist auch schneller als die HPLC-Methoden. Außerdem werden die Phasen besser aufgelöst und eindeutig identifiziert. Die Liniarität der Detektorempfindlichkeit wurde gegenüber Cannabidiol, Δ^8-Tetrahydrocannabinol, Δ^9-Tetrahydro-cannabinol und Cannabinol geprüft. Die Nachweisgrenzen liegen bei 0.55 ng, 1.20 ng, 0,69 ng bzw. 2.10 ng. Das Verfahren bietet die Möglichkeit Cannabis Produkte in einem einzigen Analysenlauf eindeutig zu identifizieren. Die Anwendung in der Fallarbeit wird beschrieben.

Se describe un método rápido de análisis de derivados del Cannabis por cromatografia de fluidos supercríticos (SFC) ligada a espectrometría de masas con detección de ionización química a presión atmosférica (APCI-MS). El método tiene unos tiempos de análisis más cortos que los de GC-MS, sin necesidad de derivatización previa. Es también más rápido que los métodos de HPLC, con mejor resolución e identificación definitiva. Se estableció la linealidad de la respuesta del detector para el cannabidiol, Δ^9 tetrahidrocannabinol, Δ^9 tetrahidrocannabinol y Cannabinol; los límites de detección en columna fueron de 0,55 ng, 1,20 ng, 0,69 ng y 2,10 ng respectivamente. La técnica ofrece los medios para identificar con seguridad los derivados cannabicos de un solo cromatograma. Se describe la aplicación en las muestras de rutina.

Key Words: Forensic science; Drugs of abuse; Supercritical fluid chromatography; Atmospheric pressure chemical ionization mass spectroscopy; *Cannabis*; Drug identification.

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Introduction

The products of *Cannabis sativa* L. account for the majority of the forensic casework samples received into forensic science laboratories for examination and analysis [1]. Their definitive identification can be achieved through the combined use of light microscopy and/or the Duquenois-Levine colour test followed by gas chromatography-mass spectroscopy (GC-MS) [2]. However, the former methods alone are insufficient for the unambiguous identification of the drug. Whilst GC-MS offers definitive identification, it suffers from long analysis times (circa 35 minutes) and the need to derivatize the sample prior to analysis [3].

The relationship of street samples of cannabis can be established using HPLC [1-4] where the quantitative ratios of the major analytes (cannabidiol, Δ^8- and Δ^9-tetrahydrocannabinol and cannabinol) (Figure 1) are all significant. However, HPLC alone does not offer definitive identification of the drug; it requires long analysis times [3,4] and suffers from short analytical column life. Gas chromatographic analysis is also employed (Alm, personal communication), but suffers from the thermal decomposition of the cannabinolic acids into their respective breakdown products [1], which, at best, can only be described as 'almost complete' [5]. This can be avoided by the use of derivatization [5], but this carries the risk of sample contamination.

Given that *cannabis* products form such a high percentage of the drug samples received into forensic science laboratories, a rapid, definitive, quantitative method is required for their analysis. Supercritical fluid chromatography (SFC) offers an analytical technique with high resolution and rapid analysis times due to the properties of supercritical fluids [6]. It has previously been employed for the analysis of only a limited range of drugs of abuse, including barbiturates [7], benzodiazepines [8], opiates [9], cocaine [10] and cannabinoid metabolites [11]. Interfaced with ultraviolet detection and atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS), the technique offers definitive identification of the analytes (Carrott M, Jones DC, Davidson G, unpublished data and [12–14]).

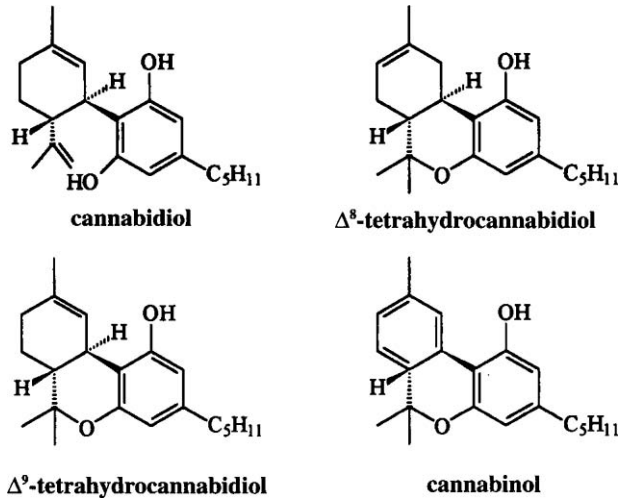

FIGURE 1 Major analytes of Cannabis.

This paper describes the use of an SFC-APCI-MS method for the analysis of *cannabis* products and the application of the method to a casework sample.

Experimental

Materials

Cannabinoid standards were purchased from Sigma Chemical Co., [Poole, Dorset, UK] as ethanol solutions at 10 mg/ml. All other chemicals and gases were of analytical reagent grade or better. Herbal drug material was chosen at random from casework samples. It was prepared for analysis by extracting the drug at 10 mg/ml in ethanol for 30 minutes at room temperature, followed by centrifuging at 4000 g for five minutes. The supernatant was subsequently analysed by GC-MS, HPLC, and SFC-APCI-MS. The same extract was used for each of the analytical methods, allowing direct comparison of the data obtained.

Prior to GC-MS analysis, the cannabinoids in the standard and sample solutions were derivatized with N,O-bistrimethylsilylacetamide (BSA). The drug standards were diluted to a concentration of 100 µg/ml in ethanol. Aliquots (100 µl) of the standard solution, or the ethanol extract of the drug material as prepared above, were dried under nitrogen in a derivatization vial. After BSA (50 µl) was added, the vial was immediately sealed and shaken, and the drug was derivatized at room temperature for five minutes. The derivatives were chromatographed directly.

Methods

Gas chromatography-mass spectroscopy (GC-MS) was carried out on a Perkin Elmer 8500 series gas chromatograph coupled to a Perkin Elmer Ion Trap Detector. Chromatography was performed [2] on a capillary BP-5 column (25 m, 0.22 mm i.d., 0.5 µm layer thickness). The injection temperature was 300°C and the transfer line temperature 290°C. The column temperature was programmed as follows: 170°C for 2 minutes, rising to 280°C at 6°C/min, holding at 280°C for 5 mins. The split ratio was 12:1 and the injection volume 1 µl. The ion trap detector scan rate was 1 scan/second, monitoring between m/z = 45 and m/z = 450, at an electronmultiplier voltage of 1700 V. Between each sample analysis, a solvent blank analysis was performed. Data was collected using Finnigan MAT ITDS software, v 4.10.

High performance liquid chromatography (HPLC) was carried out using a Perkin Elmer LC-250 solvent pump, coupled to a Perkin Elmer LC-235 diode array detector. The drug standards were diluted to a concentration of 100 µg/ml in ethanol. Chromatography was carried out directly on the diluted standard solutions [2], or the ethanol extracts of the drug, on a Spherisorb ODS-2 column (12.5 cm x 4.6 mm i.d., 5 µm particle size), in a solvent system of

methanol/acetic acid/water 85/14.2/0.8 v/v/v, pumped at a flow rate of 1.5 ml/min. The injection volume was 6 µl, the column temperature ambient, and the column eluate monitored at 230 nm. Between each sample analysis, a solvent blank analysis was performed. Data was recorded using PE Nelson Chromatography Software, v 5.1.

Supercritical fluid chromatography (SFC) coupled to atmospheric pressure chemical ionization-mass spectroscopic (APCI-MS) was performed using a Gilson packed column SFC system (Anachem, Luton) coupled to the APCI source of a Trio 2000 quadrupole mass spectrometer (VG Biotech, Altrincham). The SFC mobile phase was delivered using two Gilson piston pumps. A microprocessor-controlled Gilson 308 pump, fitted with a chiller (Anachem, Luton) to cool the pump head to -10°C, was used for delivery of SFC grade CO_2 (99.99%, BOC), and a Gilson 306 pump was used for the addition of organic modifiers to the mobile phase. Both pumps were connected to a Gilson 311C dynamic mixer to ensure homogeneity of the mobile phase. Samples were introduced using a Rheodyne 7125 injection valve fitted with a 5µl loop, and separation of the cannabinoids achieved using a 25 cm x 4.6 mm i.d. cyanopropyl silica packed column. The solvent was delivered at a flow rate of 2 ml/min, as 2% methanol in CO_2 (t=0), increasing the methanol to 7% in 15 minutes. Detection was performed using a Trio 2000 mass spectrometer fitted with an APCI source. The mass spectrometer was operated in positive ion mode with both full scan and single ion monitoring, as detailed in Table 1. Data collection was performed using LabBase Software v 2.1 (VG Biotech).

TABLE 1 APCI-MS conditions employed for SFC APCI-MS analysis of cannabinoids.

Discharge voltage		3.0 kV	
Source temperature		120°C	
Probe temperature		300°C	
Bath gas		150 $1hr^{-1}$ N_2	
Cone voltage		30, 40, 50 V as applied	
Full scan mode		50 to 400 amu in 1.5 sec	
Single ion monitoring			
Standard compound	m/z	retention time window (mins)	
		Start	End
CBD/THC	315	0	5.5
CBN	311	5.5	10
d_3-THC	318	0	5.5
Dwell time		0.3 seconds	
Span		0.4 amu	

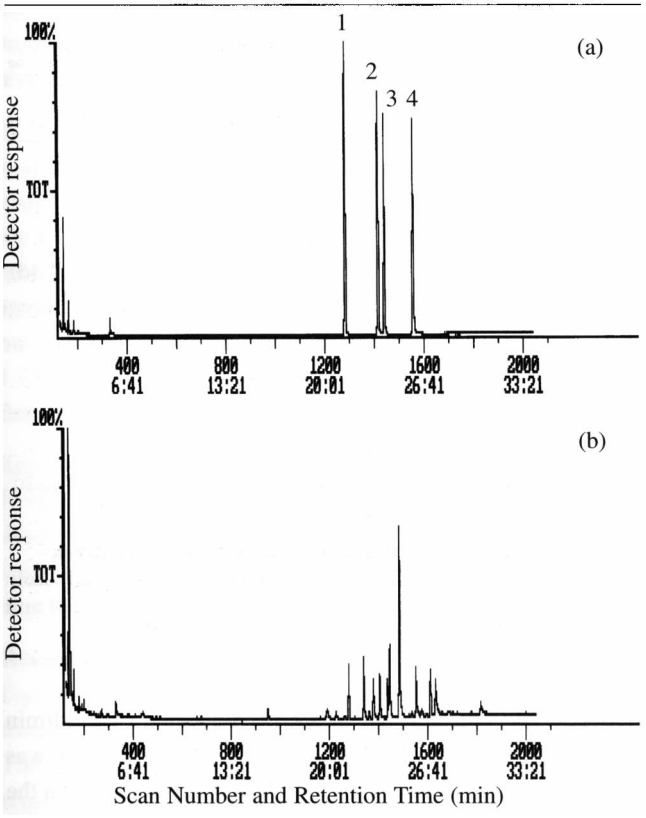

FIGURE 2 GC-MS separation of (a) cannabinoid standards and (b) components of an ethanol extract of *Cannabis*, each derivatised with N,O-bistrimethylsilylacetamide. 1. cannabidiol (1281 seconds); 2. Δ^8-tetrahydrocannabinol (1413 seconds); 3. Δ^9-tetrahydrocannabinol (1440 seconds); 4. cannabinol (1554 seconds).

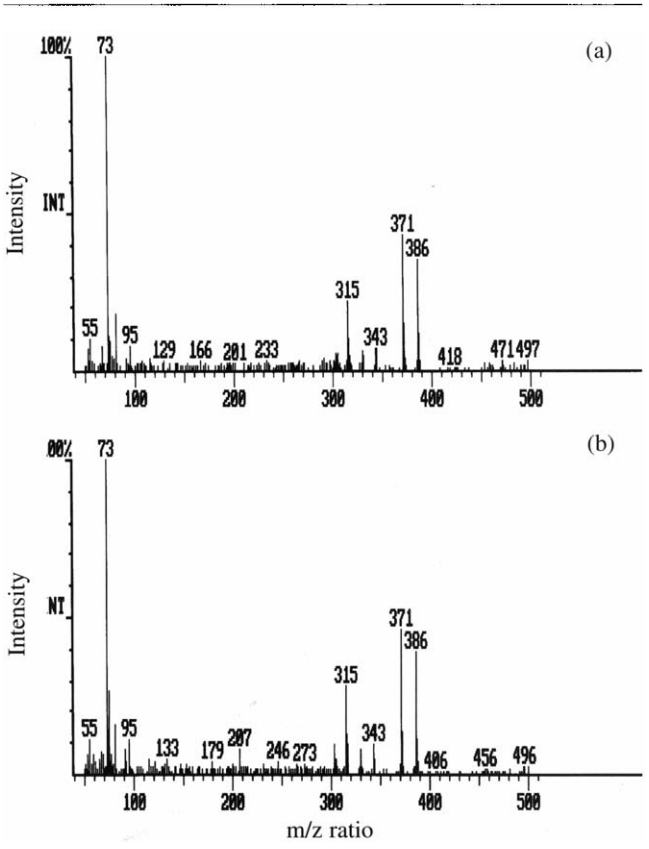

FIGURE 3 Mass spectra of derivatized (a) Δ^9-tetrahydrocannabinol standard and (b) Δ^9-tetrahydrocannabinol in cannabis.

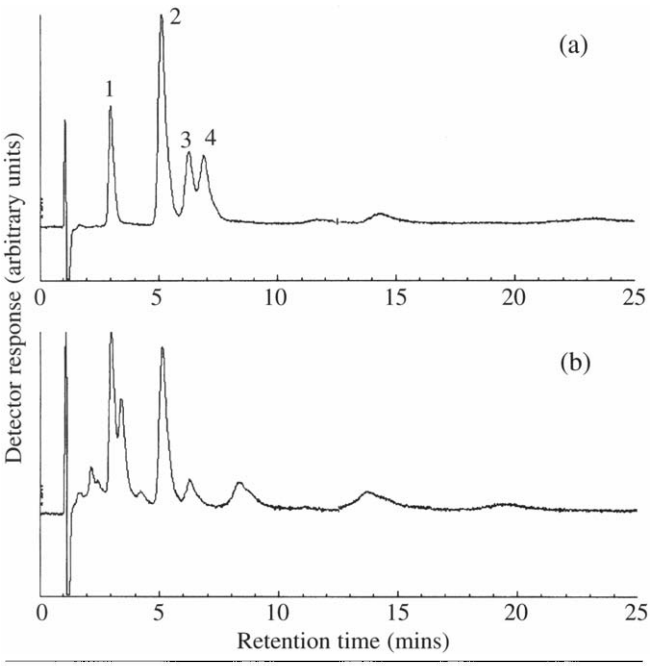

FIGURE 4 HPLC separation of (a) cannabinoid standards and (b) ethanolic extract of herbal *Cannabis sativa*. 1. cannabidiol. 2. cannabinol. 3. Δ^8-tetrahydrocannabinol. 4. Δ^9-tetrahydrocannabinol.

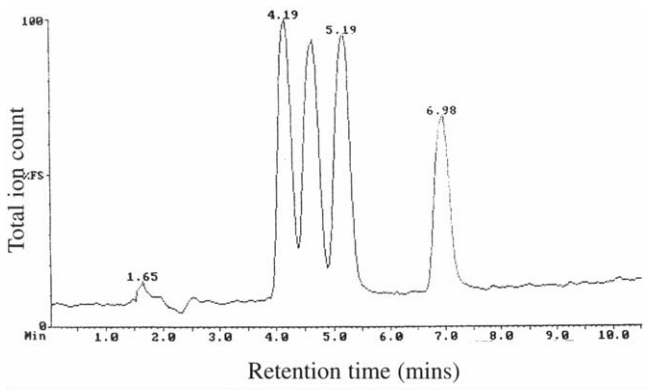

FIGURE 5 Separation of cannabinoid standards by SFC-UV-APCI-MS: cannabidiol 4.19 minutes; Δ^8-tetrahydrocannabinol 4.67 minutes; Δ^9-tetrahydrocannabinol 5.19 minutes; cannabinol 6.98 minutes.

TABLE 2 Retention times of cannabinoid standards relative to d_3-Δ^9-tetrahydrocannabinol separated by SFC-APCI-MS.

Compound	*Relative retention time*
cannabidiol	0.80
Δ^8-tetrahydrocannabinol	0.90
Δ^9-tetrahydrocannabinol	1.00
cannabinol	1.34

Interfacing SFC with APCI-MS was achieved using a drawn fused silica restrictor (30cm x 75µm id). The positions of the restrictor, probe, gas flow rates and probe temperatures had been optimised previously (Carrott M, Jones DC, Davidson G, unpublished data). The restrictor tip was drawn in a bunsen flame and cut to give an inlet pressure

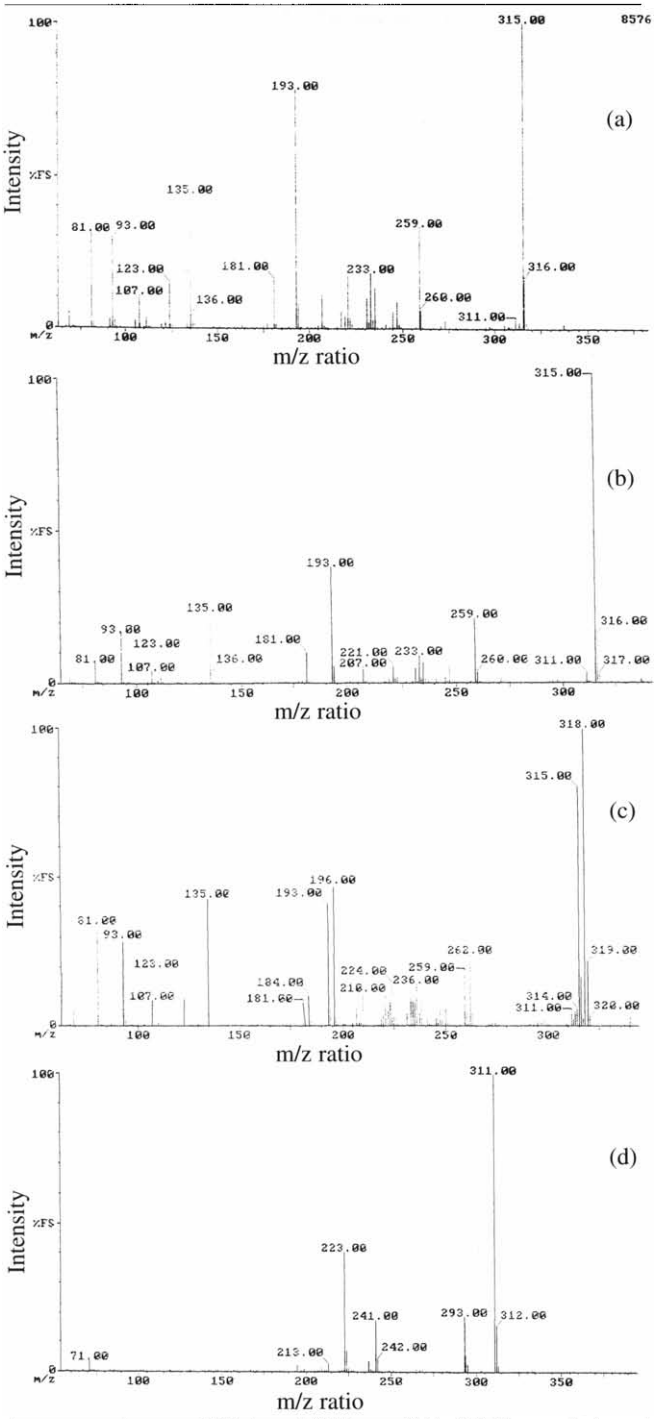

FIGURE 6 Mass spectra of cannabinoid standards separated by the SFC-UV-APCI-MS system. (a) cannabidiol; (b) Δ^8-tetrahydrocannabinol; (c) Δ^9-tetrahydrocannabinol and d_3-Δ^9-tetrahydrocannabinol; (d) cannabinol.

between 180 and 230 bar at a CO_2 flow rate of 2 ml/min when positioned in the heated probe. The restrictor was connected to the outlet of the UV detector, positioned in the heated APCI nebuliser probe, and inserted into the APCI source.

Reproducibility and linearity studies were undertaken to test whether the system would be suitable for routine analyses in operational laboratories. For both, a deuterated

TABLE 3 Retention time data for cannabinoid standards relative to d_3-Δ^9-tetrahydrocannabinol.

Sample	*Relative retention time*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Day 1, Restrictor 1							
cannabidiol	0.810	0.797	0.802	0.802	0.809		
Δ^8-tetrahydrocannabinol	0.900	0.898	0.898	0.893	0.908		
Δ^9-tetrahydrocannabinol	1.004	1.000	1.000	1.004	1.014		
cannabinol	1.342	1.348	1.356	1.348	1.363		
Day 2, Restrictor 1							
cannabidiol	0.809						
Δ^8-tetrahydrocannabinol	0.896						
Δ^9-tetrahydrocannabinol	1.006						
cannabinol	1.343						
Day 2, Restrictor 1							
cannabidiol	0.809	0.800	0.798	0.796	0.803	0.806	0.800
Δ^8-tetrahydrocannabinol	0.908	0.894	0.890	0.896	0.899	0.899	0.893
Δ^9-tetrahydrocannabinol	1.004	1.007	0.996	1.000	1.000	1.005	0.993
cannabinol	1.339	1.3354	1.350	1.349	1.343	1.342	1.352

internal standard, d_3-Δ^9-tetrahydrocannabinol, was added to all samples to a final concentration of 0.05 mg/ml. During the reproducibility studies, the relative retention times of the compounds of interest against the internal standard were recorded within a day, between days and between pressure restrictors.

In order that the system could be used for quantitative analysis and drug quantification, the linearity of detector response was established, with the MS operated in single ion monitoring mode. The relative peak areas of the protonated molecular ion of each cannabinoid were calculated against the protonated molecular ion of the deuterated internal standard, over the drug concentration range 1.25 µg/ml–125 µg/ml. From this data, the theoretical detection limit was calculated.

Application of the methods to a casework sample

Herbal cannabis material was extracted and analysed as described above. For SFC-APCI-MS, the internal standard solution was added to give a final concentration of 0.025 mg/ml.

Results and discussion.

Figure 2 demonstrates the separation by GC-MS of the trimethylsilylethers of cannabinoids in a cannabis sample. The total analysis time was 35 minutes, with further time required for equilibration of the gas chromatograph. Figure 3 shows the MS of the TMS ether of Δ^9-tetrahydrocannabinol in (a) standard solution and (b) cannabis sample.

Figure 4 shows that separation of the major cannabinoids was achieved by HPLC but only partial resolution of the two isomers of tetrahydrocannabinol was possible. The total analysis time could be up to 40 minutes, depending upon the complexity of the sample. Whilst diode array detection, which is frequently employed with HPLC analysis, supports the identification of the cannabis, it cannot be used as a definitive identification.

Using the SFC-UV-APCI-MS system described, the cannabinoids were separated in less than eight minutes (Figure 5). The absolute retention times in minutes of the compounds were cannabidiol 4.19, Δ^8-tetrahydrocannabinol 4.67, Δ^9-tetrahydrocannabinol 5.19 and cannabinol 6.98. Further, definitive identification of the cannabinoids was possible through the use of a combination of retention time relative to the deuterated internal standard (Table 2) and the mass spectra at a cone voltage of 50V (Figure 6). The relative retention times of the cannabinoids to the internal standard were stable within a day, between days and between restrictors (ANOVA, $p<0.05$) (Table 3).

In terms of resolution of the compounds, the separation was not as good as for GC-MS, but was an improvement upon that achieved by HPLC. However, the sample derivatization required for GC-MS was not required for SFC-APCI-MS. The peak symmetries as measured at half height gave values of 1.2, 1.0, 1.0 and 1.2 for cannabidiol, Δ^8 -tetrahydrocannabinol, Δ^9-tetrahydrocannabinol and cannabinol respectively, indicating that symmetrical peaks could be achieved using this system.

The APCI-MS spectra at low cone voltage showed the characteristic protonated molecular ion expected for each of the analytes. Obviously, this does not provide definitive identification of these compounds. By increasing the cone voltage, it was possible to produce controllable fragmentation

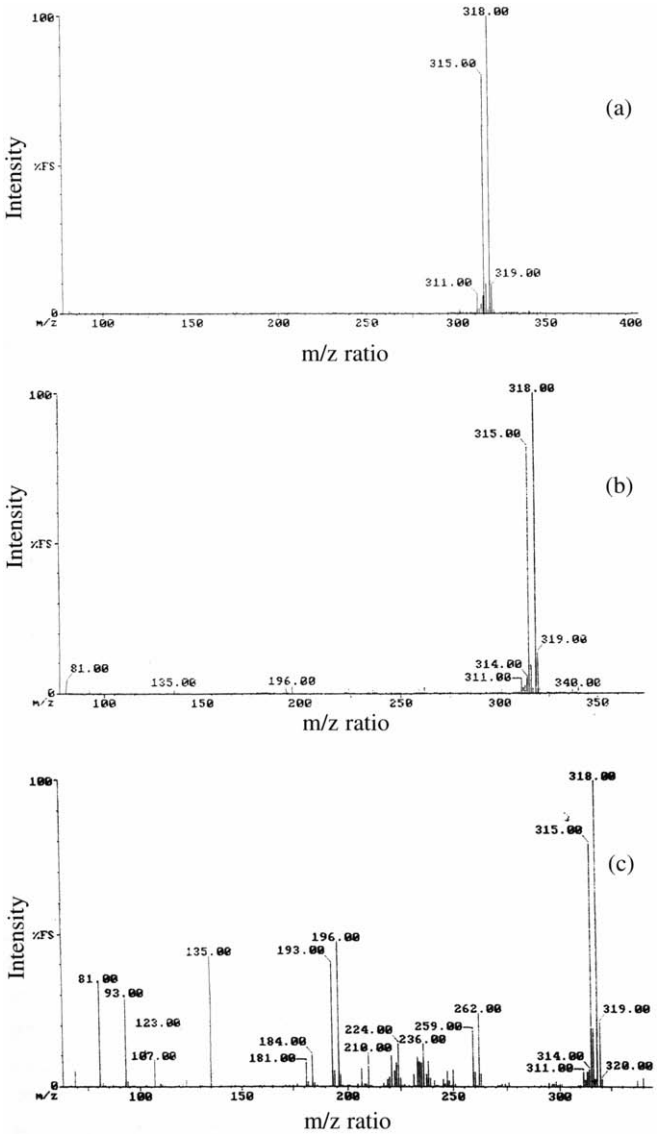

FIGURE 7 Fragmentation pattern for Δ^9-tetrahydrocannabinol and d_3-Δ^9-tetrahydrocannabinol under different applied cone voltages. (a) 30V; (b) 40V; (c) 50V.

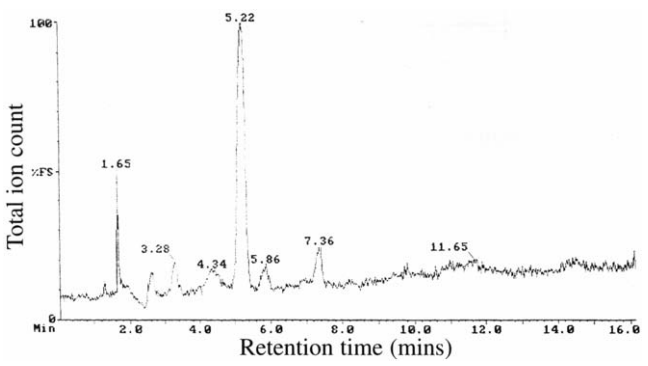

FIGURE 8 Total ion count analysis by SFC-UV-APCI-MS system of a herbal cannabis sample.

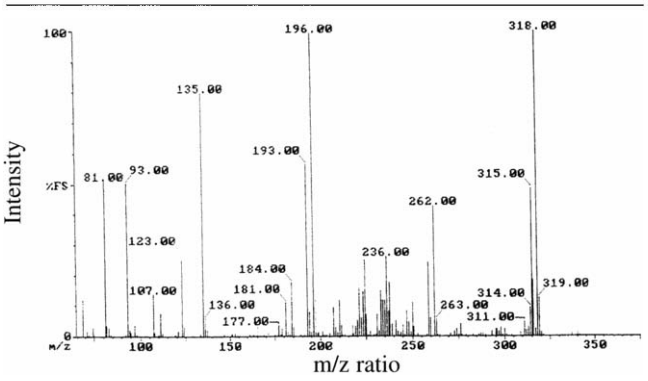

FIGURE 9 Mass spectrum of Δ^9-tetrahydrocannabinol in the presence of d_3-Δ^9-tetrahydrocannabinol.

and to obtain structural information, as exemplified for Δ^9-tetrahydrocannabinol (Figure 7). Thus a combination of retention time data and mass spectrometry can be used to differentiate between the cannabinoids tested in this study.

Using single ion monitoring, linear responses to the four cannabinoids over the concentration range tested were obtained (Table 4). The theoretical detection limits for mass of cannabinoid on column were calculated as being 0.55 ng, 1.20 ng, 0.69 ng and 2.10 ng for cannabidiol, Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol and cannabinol respectively. These detection limits allow analysis of casework samples and drug profiling materials.

Application to casework samples

When applied to casework samples, the SFC-APCI-MS technique allowed the definitive identification of the Δ^9-tetrahydrocannabinol (Figures 8 and 9) in the herbal cannabis sample, illustrating that the methods could be applied to casework samples.

Conclusions

The SFC-APCI-MS method offers a rapid, definitive technique for the quantitative analysis of cannabis samples. It is

TABLE 4 Linearity data and detection limits for cannabinoid standards separated by SFC-APCI-MS.

Compound	*Regression equation*	*r^2 value*	*p value*	*Detection limit (on column) (ng)*
Cannabidiol	Y=0.0128 X + 0.039	0.999	<0.05	0.55
Δ^8-tetrahydrocannabinol	Y=0.0208 X + 0.079	0.997	<0.05	1.20
Δ^9-tetrahydrocannabinol	Y=0.0208 X + 0.079	0.997	<0.05	0.69
Cannabinol	Y=0.0208 X + 0.079	0.997	<0.05	2.10

more rapid than GC-MS, and does not require sample derivatization, reducing the risk of sample contamination. It is also more rapid than HPLC and offers greater resolution, allowing more samples to be analysed per unit time. Furthermore, it does not create the large volumes of liquid waste that require disposal. The SFC-APCI-MS method can be employed on casework samples without difficulty and is sufficiently sensitive for the analysis of bulk samples and trace analyses. It therefore offers an attractive alternative to GC-MS for the analysis of cannabis samples.

References

1. Gough TA, editor. The analysis of drugs of abuse. Chichester: John Wiley & Sons, 1991.
2. Anon. Recommended methods for testing of *Cannabis*. Vienna: United Nations Drug Control Programme, 1992.
3. Gillan R, Cole MD, Linacre A, Thorpe JW and Watson ND. Comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids–a preliminary study. Science and Justice 1995; 35: 169–177.
4. Lehmann T and Brenneisen R. High performance liquid chromatographic profiling of *Cannabis* products. Journal of Liquid Chromatography 1995; 18: 689–700.
5. Heuzer H. The use of gas chromatography for the detection and quantitation of abused drugs. In: Gough TA, editor. The analysis of drugs of abuse. Chichester: John Wiley & Sons, 1991.
6. Bevan CD and Marshall PS. The use of supercritical fluids in the isolation of natural products. Natural Product Reports 1994; 11: 451-466.
7. Smith RM and Sanagi MM. Supercritical fluid chromatography of barbiturates. Journal of Chromatography 1989; 481: 63–69.
8. Smith RM and Sanagi MM. Packed column supercritical fluid chromatography of benzodiazepines. Journal of Chromatography 1989: 483; 51–61.
9. Janicot JL, Caude M. and Rosset R. Separation of opium alkaloids by carbon dioxide subcritical and supercritical fluid chromatography with packed columns – application to the quantitative analysis of poppy straw extracts. Journal of Chromatography 1988; 437: 351–364.
10. Mackay GA and Reed GD. The application of capillary SFC, packed column SFC and capillary SFC-MS in the analysis of controlled drugs. Journal of High Resolution Chromatography 1991; 14: 537–541.
11. Later DW, Richter BE, Knowles DE and Andersen MR. Analysis of various classes of drugs by capillary supercritical chromatography. Journal of Chromatographic Science 1986; 24: 249–253.
12. Olesik SV. Recent advances in supercritical fluid chromatography mass spectrometry. Journal of High Resolution Chromatography 1991; 14: 5.
13. Huang E, Henion J and Covey TR. Packed column supercritical fluid chromatography mass spectrometry and supercritical fluid chromatography tandem mass spectrometry with ionization at atmospheric pressure. Journal of Chromatography 1990; 511: 257–270.
14. Tyrefors LN, Moulder RX. and Markides KE. Interface for open tubular column supercritical fluid chromatography atmospheric pressure chemical ionisation mass spectrometry. Analytical Chemistry 1993; 65: 2835–2840.