CHROM. 7977

DETECTION OF LYSERGIC ACID DIETHYLAMIDE, Δ^9-TETRAHYDRO-CANNABINOL AND RELATED COMPOUNDS BY PLASMA CHROMATOGRAPHY

F. W. KARASEK[*], D. E. KARASEK and S. H. KIM

Department of Chemistry, University of Waterloo, Waterloo, Ontario (Canada)

(Received September 30th, 1974)

SUMMARY

Plasma chromatography as a method for ultratrace qualitative and quantitative detection of organic compounds is especially well suited for detection of gas chromatographic effluents. The optimum range of sample quantity is 10^{-6} to 10^{-12} g for detection and identification of a compound by use of its characteristic positive and negative mobility spectra. The type of reference mobility spectra produced by alkanes, aromatics, esters, halogenated compounds, nitrogenated compounds and organic acids have been previously reported. This study presents the reference mobility spectra produced for lysergic acid diethylamide (LSD), Δ^9-tetrahydrocannabinol (Δ^9-THC), digitoxigenin and several biochemical compounds of research significance. LSD and Δ^9-THC in a mixture can be detected and identified by plasma chromatography positive mobility spectra in quantities of 10^{-7} g or less. All the compounds investigated in this study display strong MH^+ ions along with other ions primarily of the type $(M)NO^+$, $(M)_2H^+$. None of these compounds exhibits negative mobility spectra.

INTRODUCTION

Operating at atmospheric pressure, plasma chromatography gives qualitative and quantitative analysis for ultratrace amounts of a sample. The technique is based on observing the positive and negative ion mobility spectra which originate by an ion–molecule reaction between the trace amount of organic compound and the generated reactant ions or electrons. Ions for the reaction are generated by the action of 60-KeV electrons emitted from a ^{63}Ni foil into a purified nitrogen or air carrier gas containing a trace of water vapor. In nitrogen, the primary ions formed undergo a series of reaction steps to evolve the stable species $(H_2O)_nH^+$ and $(H_2O)_nNO^+$ ions, whose relative abundance and value of n depend upon water concentration and temperature. The negative particles are low-energy (*ca.* 0.5 eV) electrons. When air is

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[*] To whom correspondence should be addressed.

used as a carrier gas, $(H_2O)_nO_2^-$ ions are added to these groups. The resultant product ions from the ion–molecule reactions are separated in a coupled ion-drift tube to give positive and negative spectra characteristic of organic molecules involved. Both the instrumentation and technique have been described[1–6].

In the negative mode, the instrument functions as an electron capture detector (ECD) that produces a qualitative spectrum of the compound in a gas chromatographic (GC) peak. A number of studies of the ECD and its mechanism have demonstrated the existence of dissociative electron capture for monohalogenated benzenes[7], both dissociative and associative electron capture for substituted benzenes and polychlorinated biphenyl compounds[8–10], and halide ion formation by dissociative capture for the alkyl halides[11]. According to these data, the negative ion mobility spectra obtained are simple and characteristic, and even isomers such as the three monochlorobenzenes[9] and three isomeric phthalic acids[12] can be distinguished.

Whereas only compounds that give a response in the ECD give a response in the negative mode using nitrogen carrier gas, all compounds studied so far exhibit a response in the positive mode. In most cases, the intensities of the positive spectra are greater than those of the corresponding negative spectra. The positive reactant ions species of type $(H_2O)_nH^+$ and $(H_2O)_nNO^+$ readily react with trace molecules to give protonated molecular ions[13,14] and very simple fragmentation ions similar to those found in chemical ionization mass spectrometry (CI-MS)[15,16] for alkane and aromatic compounds or more complex ion-molecules of the form $(M)_x(H_2O)_yH^+$ and $(M)_x(H_2O)_yNO^+$ for polar compounds like alcohols, acids and ethers[12,15].

The plasma chromatograph can be used either directly to detect and identify single compounds, or as a very sensitive qualitative detector for GC effluents. Development of both techniques requires the determination of reference mobility spectra for many classes of compounds. Systematic studies of the positive and negative ion mobility spectra (necessary as reference spectra for use in detection of GC effluents) have included those of oxygenated compounds[2], monohalogenated benzene[7], 1-haloalkanes[11] and n-alkanes[17]. The present study was undertaken as part of a continuing effort to provide qualitative plasma chromatographic data for different classes of compounds. New reference spectra are presented for several types of biochemical compounds including drugs like Δ^9-tetrahydrocannabinol (Δ^9-THC), lysergic acid diethylamide (LSD) and digitoxigenin.

EXPERIMENTAL

Instrumentation

A BETA-VI model plasma chromatograph (Franklin GNO, West Palm Beach, Fla., U.S.A.) was used in this study. The basic design and operating characteristics have been described[4–8]. Because of the high sensitivity, the sample must be in the 10^{-6}—10^{-12} g range in order not to saturate the instrument and produce uninterpretable mobility spectra from complex ion-molecule–molecule reactions.

All plasma chromatographic data obtained in this study were taken at the following conditions, unless otherwise shown in figure captions: carrier-gas flow-rate, 100 ml/min; drift-gas flow-rate, 380 ml/min; ion–molecule reactor length, 6.0 cm; ion drift space length, 6.0 cm; electric field gradient, 214 V/cm; pressure, 729.3–735.4 torr; carrier and drift gas, nitrogen (Linde, high purity 99.996 %); injection- and scan-

gate widths, 0.5 msec; temperature, 200 °C; electrometer sensitivity, 30 mV f.s.d.

Reagents

LSD (Sandozlot) and Δ^9-THC were provided Dr. R. A. Graham (Pharmaceutical Chemistry Division, Ministry of Health and Welfare, Ottawa, Canada). L-Cystinyl-bis-L-alanine, S-(1,2-dicarboxyethyl)cysteine and hydantoin-5-propionic acid were provided by Dr. A. Niederwieser (Universitäts Kinderklinik, Zürich, Switzerland). Digitoxigenin was provided by Dr. D. M. Karasek (University of Oklahoma, Medical School, Oklahoma City, Okla., U.S.A.).

Procedure

The amount of sample used in this study is 10^{-7}–10^{-9} g. All the samples were introduced into the inlet tube using a platinum wire onto which sample solutions (methanol or ethanol solvent) are dispensed by a microliter syringe and the solvent is air evaporated prior to sample introduction. Samples were introduced during scan as shown in Figs. 1–3 to obtain maximum sensitivity. Observation of the abundance of reactant ions is useful for following the reaction between the reactant ions and trace molecules of the sample. All sample quantities reported are quantities injected into the instrument. However, peaks observed in the mobility spectra may represent sample concentrations several orders of magnitude lower, because the sample is diluted exponentially with time by instrument carrier gas. Examination of the positive reactant ion mobility spectra for return to its intensity prior to sample injection permits one
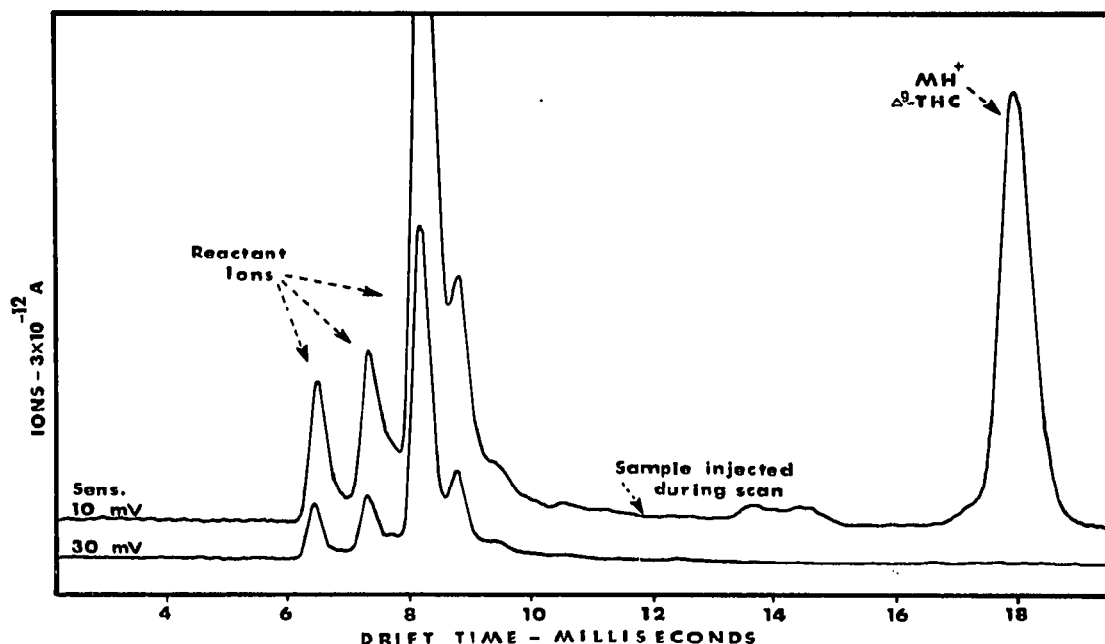

Fig. 1. Positive mobility spectra of Δ^9-THC at sample of 10^{-8} g; injection- and scan-gate widths, 0.2 msec; electric field gradient, 250 V/cm.
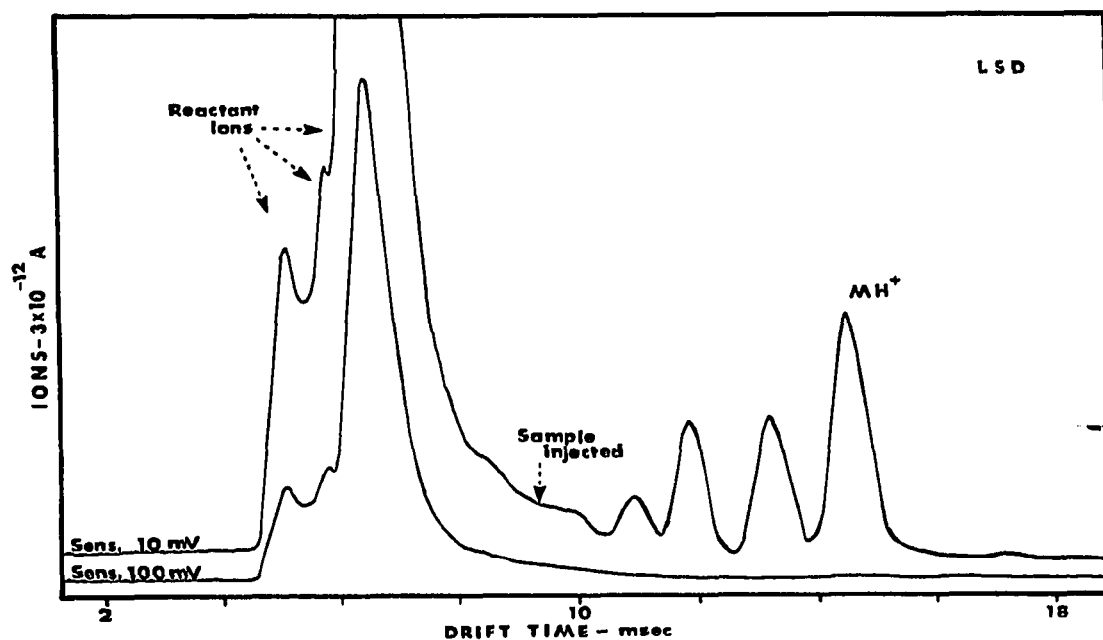
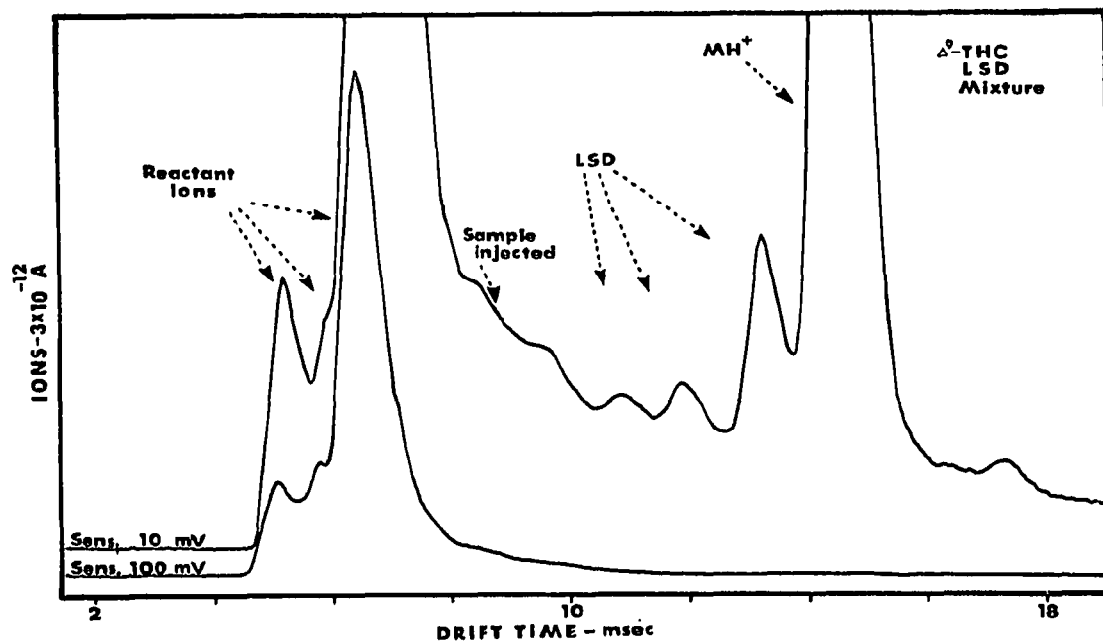Fig. 2. Positive mobility spectra of an LSD sample of $2 \cdot 10^{-7}$ g.

Fig. 3. Positive mobility spectra of the mixture of an LSD and Δ^9-THC sample of $2 \cdot 10^{-7}$ g.

TABLE I

CALCULATED K_0 VALUES FOR POSITIVE IONIC SPECIES OBSERVED IN PLASMA CHROMATOGRAPHIC SPECTRA

Compound	K_0 $(cm^2 \cdot V^{-1} \cdot sec^{-1})$	Compound	K_0 $(cm^2 \cdot V^{-1} \cdot sec^{-1})$
L-Cystinyl-bis-L-alanine	1.16	Hydantoin-5-propionic acid	1.50
	1.08		1.38
	1.00		1.22
	0.90		
		Δ^9-THC	1.06
Digitoxigenin	1.42		
	1.18	S-(1,2-Dicarboxyethyl)-	1.26
	1.13	cysteine	1.18
	0.91		1.08
			1.00
LSD	1.40		0.86
	1.30		
	1.16		
	1.05		
	0.90		

to determine when the instrument is ready for another sample injection. The reduced mobility, K_0, values are calculated from the equation

$$K_0 = \frac{6.55}{\tau T} \cdot \frac{p}{760}$$

where τ = drift time (sec); T = absolute temperature (°K); p = pressure (torr); the factor 6.55 incorporates cell length (6 cm), electric field gradient (250 V/cm) and correction to 273 °K. All K_0 values reported have a standard deviation of $\pm$ 0.02. Table I lists accurately measured K_0 values for the ionic species observed, while the data in Fig. 4 serve to show relative ion abundances in the observed spectra.

RESULTS AND DISCUSSION

The positive and negative mobility spectra of Δ^9-THC, LSD and other related biochemical compounds were obtained. Whereas all the compounds produced simple and characteristic positive mobility spectra, none of these compounds shows a negative mobility spectrum. In the positive mode these compounds react with the positive reactant ions to give ionic species MH^+, $(M)NO^+$, $M(H_2O)_nH^+$ and $M(H_2O)_nNO^+$.

As is typically found in the mobility spectra of drugs, the compounds investigated in this study show strong MH^+ ion with lesser abundant ions of $(M-R)H^+$ (R = alkyl, S or functional group), $(M)NO^+$, and $(M)_2H^+$ types, as shown in Figs. 1, 2 and 4. The positive mobility spectrum of Δ^9-THC displays a very strong single peak, which is assigned as MH^+ ion, at K_0 = 1.06, as shown in Fig. 1. This agrees with the result indicated previously by Karasek[6], and the result of a CI-MS study by Finkle et al.[18]. As shown in Figs. 2 and 4, LSD also exhibits an intense MH^+ ion peak at K_0 = 1.05, along with three other fragment-ion peaks at K_0 = 1.16, 1.30 and 1.40, respectively, and an unidentified weaker ion peak at a higher mass mobility
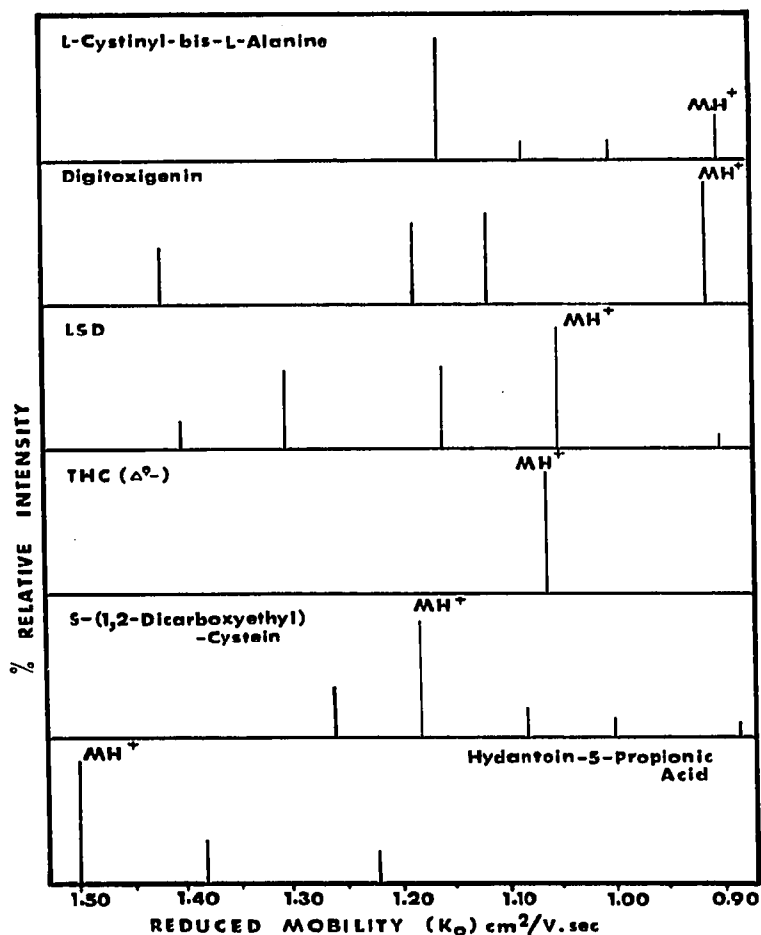
Fig. 4. Reference spectra of normalized plots of positive ionic species intensities *vs.* K_0 for the LSD, Δ^9-THC and related compounds. LSD and Δ^9-THC are taken at 200°C; the others are at 150–155°C range.

value of $K_0 = 0.9$. The ion peak at $K_0 = 1.16$ appears to be $(M-43)H^+$ ion, as Finkle *et al.*[18] and Nigam and Holmes[19] have reported in their CI-MS and electron ionization mass spectrometric (EI-MS) studies, respectively, presumably by the loss of a $CH_3-N=CH_2$ group. The ion peak at $K_0 = 1.30$ can be assigned to a protonated ion formed by losing the amido carbonyl group and tertiary amino group. The ion peak with mild intensity at $K_0 = 1.40$ could be $(M-115)H^+$, which corresponds to the protonated form after the loss of CH_3 and $(C_2H_5)_2NO$ groups. These assignments agree well with the results obtained by Nigam and Holmes[19] in their EI-MS study for LSD.

To investigate the detection and identification possibility of Δ^9-THC and LSD from an unknown sample mixture, the positive ion mobility spectra were obtained using the mixture of Δ^9-THC and LSD. Fig. 3 shows the positive ion mobility spectra from the sample $2\cdot10^{-7}$ g of the mixture of Δ^9-THC and LSD in a 1:25 weight ratio.

The MH$^+$ ion of Δ^9-THC at $K_0 = 1.06$ and the MH$^+$ ion of LSD at $K_0 = 1.05$ are overlapped because of the close K_0 values. However, by use of the fragment ions of LSD (see Fig. 3), one can detect and identify the LSD present in a mixture with Δ^9-THC.

L-Cystinyl-bis-L-alanine, digitoxigenin, S-(1,2-dicarboxyethyl)cysteine, hydantoin-5-propionic acid, compounds whose analysis is important to certain biochemical research[20], were also investigated in this study. A specific method for rapid measurement of digitoxigenin in clinical work is especially important. The normalized relative intensities of the positive ion mobility spectra of all these compounds are shown in Fig. 4. All the MH$^+$ ions of these compounds are the most prominent in the spectra, except for L-cystinyl-bis-L-alanine. L-(1,2-Dicarboxyethyl)cysteine displays the MH$^+$ ion as the most prominent peak at $K_0 = 1.18$, and the weak peaks of higher mass than molecular weight appear to be (M)NO$^+$, M(H$_2$O)$_n$H$^+$ ($n = 3$ or 4) and (M)$_2$H$^+$ at $K_0 = 1.08$, $K_0 = 1.00$ and $K_0 = 0.86$, respectively. Hydantoin-5-propionic acid also exhibits its MH$^+$ ion as the most prominent ion peak at $K_0 = 1.50$ with two other fairly strong peaks, which appear to be (M)NO$^+$ and (M)$_2$H$^+$ at $K_0 = 1.38$ and $K_0 = 1.22$. Only L-cystinyl-bis-L-alanine does not show the MH$^+$ ion as the most prominent peak. This suggests that this compound may be thermally rather unstable and easily gives a fragment-ion peak presumably by cleavage of the sulphur–sulphur bond to give an ion with a K_0 value of 1.16 as the most prominent peak. Digitoxigenin shows the MH$^+$ ion as the most prominent peak at $K_0 = 0.91$ with three other fairly strong fragment-ion peaks. All the mass assignments in this study reasonably agree with the curve of mass *vs.* reduced mobility for oxygen-containing compounds[6]. Because it is the overall spectral pattern that leads to identification of the compound, no attempt was made to postulate the identity of a number of the ions shown in Fig. 4. Based on the data presented here, very sensitive and specific analytical methods for these compounds can be developed using the plasma chromatographic technique.

ACKNOWLEDGEMENTS

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