Journal of Chromatography, 514 (1990) 377–382
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 568

Note

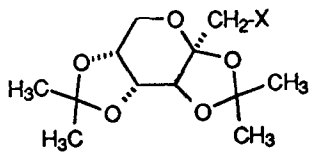
Indirect thin-layer chromatography–fast atom bombardment and chemical ionization mass spectrometry determination of carbohydrates utilizing simple and rapid microtransfer techniques

GARY W. CALDWELL*, JOHN A. MASUCCI and WILLIAM J. JONES
Department of Chemical Research, Janssen Research Foundation at McNeil Pharmaceutical, Spring House, PA 19477 (U.S.A.)
(First received January 22nd, 1990; revised manuscript received May 15th, 1990)

Thin-layer chromatography (TLC) and mass spectrometry (MS) can be combined in both direct and indirect modes[1]. The TLC plate can be sampled directly by MS. In this case, two approaches can be taken: a sample can be analyzed directly from a TLC plate mounted inside the ion source, using for example secondary ion mass spectrometry (SIMS)[2–5], or laser mass spectrometry[6,7] for the ionization process, or the sample can be desorbed from the TLC plate and transferred to the ion source through a heated line[8]. In some cases, small segments of the TLC plate are cut out and attached to an MS insertion probe[9,10]. While direct-mode techniques offer many advantages over indirect methods, they do have some disadvantages such as differential compound sensitivity, selection of suitable matrix, prevention of diffusion of spots on the TLC plate, and the unavailability of direct TLC–MS accessories and/or dedicated TLC–MS instrumentation. The indirect mode requires that the TLC spot (analyte plus adsorbent) be removed from the plate and inserted into the MS ion source or that the sample be eluted from the TLC adsorbent with suitable organic solvents, evaporated and the resulting extract analyzed by standard MS techniques[11]. Thus, the usefulness of indirect TLC–MS techniques [fast atom bombardment (FAB), chemical ionization (CI) or electron ionization (EI)] depend heavily on efficient microtransfer techniques.

We wish to communicate simple and rapid microtransfer techniques for indirect TLC–FAB-MS and TLC–CI- (or EI-)MS. Various techniques have been described in the literature for performing these operations on the microscale. For example, studies using TLC–FAB-MS established detection limits of approximately 20 μg for lasalocid, septamycin and monensin when analyte was still deposited on the TLC adsorbent[12]. Their microtransfer technique involved covering the tip of the FAB probe tip with double-faced masking tape. The above compounds were sampled by pressing the probe tip against the TLC spot of interest and acquiring FAB mass spectra in the usual manner. Microtransfer elution approaches have included more elaborate systems[13].

0021-9673/90/$03.50



	X	Molecular Mass (amu)
1	OH	260
2	OSO_2NH_2	339
3	$OC(O)NH_2$	303

Fig. 1. Structure and molecular mass of 2,3:4,5-bis-O-(1-methylethylidene)-β-D-fructopyranose (**1**), sulfamate (**2**) and carbamate (**3**).

For example, these devices consist of a filtration section, a collector section and a suction pump. We have developed microtransfer techniques which require no special equipment and are much simpler than any previously reported method.

EXPERIMENTAL

Materials

The carbohydrates 2,3:4,5-bis-O-(1-methylethylidene)-β-D-fructopyranose (**1**), sulfamate (**2**) and carbamate (**3**) derivatives were synthesized according to the literature[14]. The structures and molecular masses are given in Fig. 1. All solvents used were HPLC grade, other materials were of laboratory grade and used as purchased. Common capillary tubes (90 × 1.5–1.8 mm I.D.) and borosilicate glass disposable pasteur pipettes (14 cm) were used.

Preparation of TLC plates

The TLC chromatographic analysis was performed on glass plates coated with silica gel (Whatman MK6F 60A 7.6 × 2.5 cm; Whatman, Clifton, NJ, U.S.A.). A standard mixture (5 mg/ml) of compounds **1**–**3** was spotted in two separate rows and developed. The mobile phase was cyclohexane–isopropanol (5:1) with R_F values for compounds **1** (0.60), **2** (0.48) and **3** (0.40). After development, one side of the TLC plate was covered while the other side was charred with sulphuric acid for visualization. The covered TLC spots were used for TLC–MS analysis.

Equipment

A VG 7070E mass spectrometer (VG Analytical, Manchester, U.K.) was utilized for obtaining FAB-MS data with 3-mercapto-1,2-propanediol (thioglycerol) as the matrix and argon as the gas for the FAB gun. The acceleration voltage was 5 kV with a mass range of 50–1000 daltons. A Finnigan 3300 or the VG 7070E mass spectrometer was utilized in the CI mode (ammonia) over a mass range of 50–700 daltons.

Microtransfer method for TLC–FAB-MS

A microtransfer method was developed by which the above compounds could be sampled by indirect TLC–FAB-MS. In general, a small amount of thioglycerol was placed on the end of a capillary tube. By touching the capillary tube to the TLC spot of interest, the silica gel was softened and removed (sample plus adsorbent). The silica adsorbent containing analyte was deposited directly on the FAB probe tip and analyzed. Additional thioglycerol was added (1 μl) prior to inserting the FAB probe into the MS.

Microtransfer method for TLC–CI- or EI-MS

The TLC–CI- (or EI-)MS microtransfer method involved a simple one-step

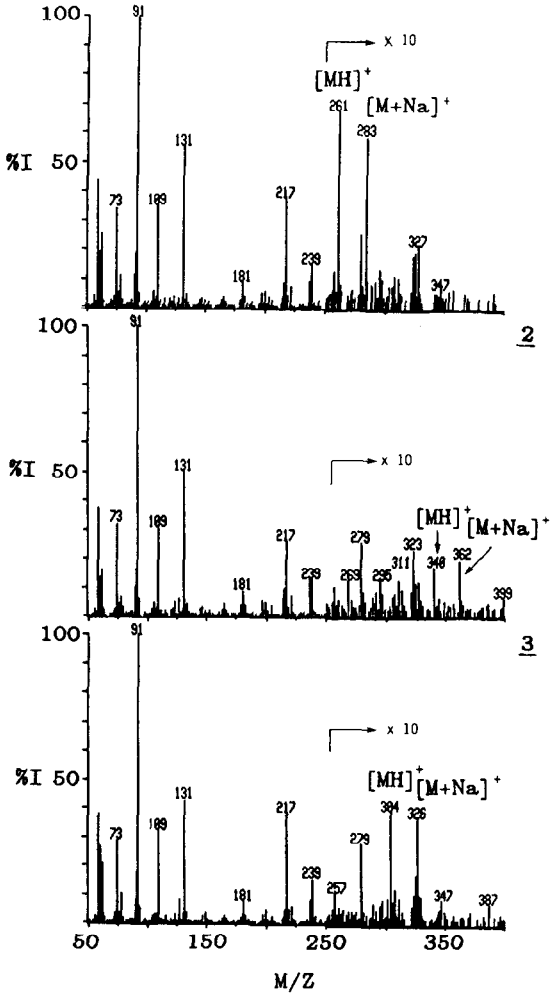


Fig. 2. The TLC–FAB-MS (thioglycerol) spectra of compounds **1–3** are shown. Spectra were obtained from a developed TLC plate (silica gel) where **1–3** were spotted at 5 μg each. The compound plus adsorbent were removed from the TLC plate and inserted directly into the mass spectrometer. I = Relative intensity.

extraction prior to MS analysis. This method involved utilizing a disposable pipette to scrape the TLC spot of interest. After scraping, the sample and adsorbent were located in the tip of the pipette and were transferred to a vial. By holding the vial at approximately a 45° angle and adding approximately 25–50 μl of a chloroform–methanol (50:50) mixture, the vial was slowly rotated in such a manner that the adsorbent remained at the top of the vial and the solution containing the compound of interest was at the bottom of the vial. The pipette was again used to remove the solution by capillary action. This solution, which contained the sample plus a small amount of adsorbent, was placed on an MS insertion probe, evaporated and analyzed.

RESULTS AND DISCUSSION

A glass-backed silica gel TLC plate was used to chromatographically separate a mixture of carbohydrates **1–3**. The TLC–FAB-MS results indicated that spectra of the above compounds could be obtained with reasonable sensitivity at 5 μg per spot (Fig. 2). The $[M + H]^+$ and $[M + Na]^+$ ions were clearly observed in all spectra and could be used for molecular-mass verification. The remaining ions in Fig. 2 are due primarily to the thioglycerol matrix as evident by comparing these ions to background ions in Fig. 3 (top). Background ions from the TLC adsorbent were not observed. The

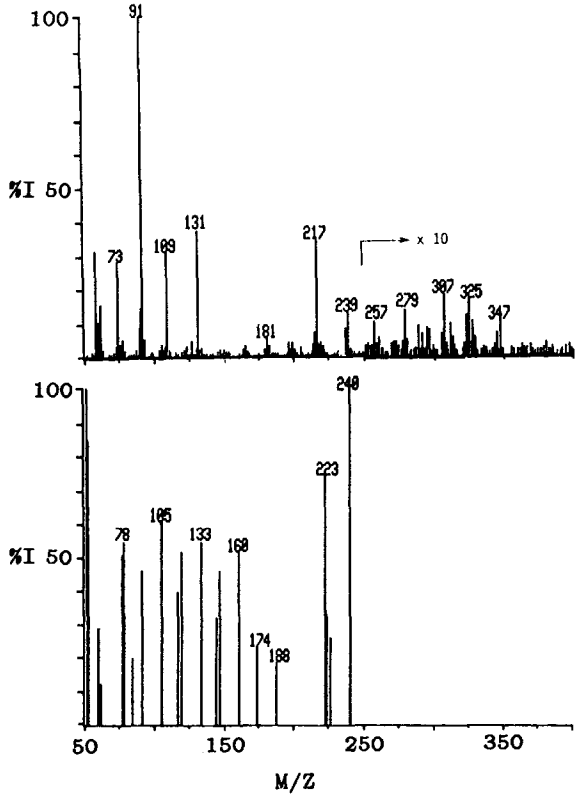


Fig. 3. The background ions are shown for TLC–FAB-MS (top, thioglycerol) and TLC–CI-MS (bottom, ammonia).

TLC–CI-MS (ammonia) results indicated that the $[M + NH_4]^+$ ion could be clearly observed at 5 μg per spot for compounds **1–3** (Fig. 4) along with fragment ions. The base peak was *m/z* 278. The *m/z* ion is probably produced for compounds **2** and **3** by ammonia substitution reactions or thermal decomposition, *i.e.*, **2** to **1** and **3** to **1**. Background ions from the TLC adsorbent were not observed (Fig. 3, bottom).

It is significant to note that the TLC plates utilized are those commonly used by organic chemists for monitoring reactions. Thus no special information is required by the chemist to submit samples for MS analysis. It is also important to note that the small amount of silica which is deposited on the probe tip for TLC–FAB-MS or TLC–CI-MS remains primarily on the tip and is removed after MS analysis. Therefore no additional MS source cleaning is required for μg-type analysis.

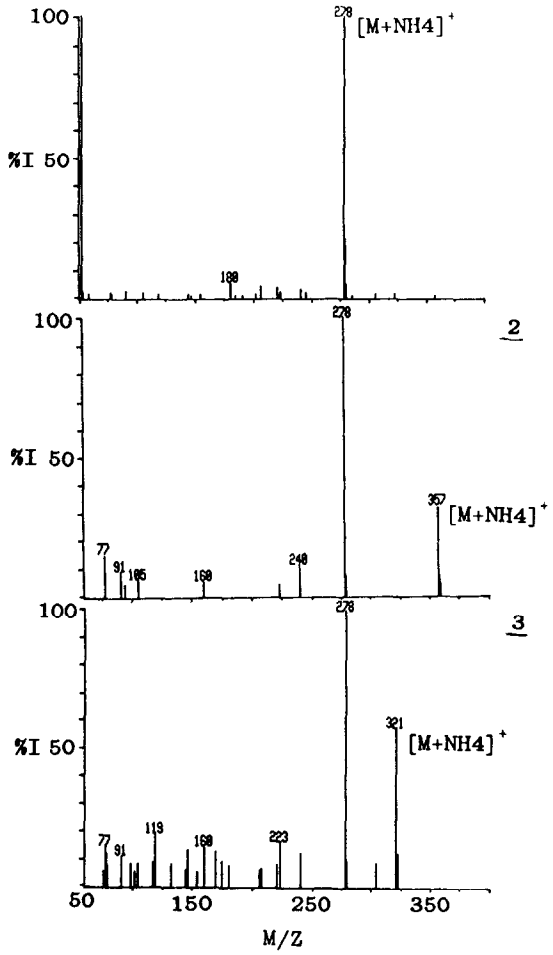


Fig. 4. The TLC–CI-MS (ammonia) spectra of compounds **1–3** are shown. Spectra were obtained from a developed TLC plate (silica gel) where **1–3** were spotted at 5 μg each. The compounds were eluted from the adsorbent prior to CI analysis.

CONCLUSION

We have developed very simple microtransfer techniques for TLC–MS which allow for rapid turn-around-times (2 min per spot) and efficient throughput of samples. In fact, we analyze approximately 400 TLC plates per year with these methods. Useful MS data can be obtained with a high degree of confidence for a wide range of samples and problems when both TLC–FAB-MS and TLC–CI-MS are used in combination. We have found that these microtransfer methods can be applied to most compounds that are separated on TLC plates.

REFERENCES

1 C. F. Poole and S. K. Poole, *J. Chromatogr.*, 492 (1989) 539–584.
2 K. Masuda, K. Harada, M. Suzuki, H. Oka, N. Kawamura and M. Yamada, *Org. Mass Spectrom.*, 24 (1989) 74–75.
3 K. L. Busch, *J. Planar Chromatogr.*, 2 (1989) 355.
4 K. L. Busch, *Trends Anal. Chem.*, 6 (1987) 95–100.
5 G. C. DiDonato and K. L. Busch, *Anal. Chem.*, 58 (1986) 3231–3232.
6 A. J. Kubis, K. V. Somayajula, A. G. Sharkey and D. M. Hercules, *Anal. Chem.*, 61 (1989) 2516–2523.
7 F. P. Novak and D. M. Hercules, *Anal. Lett.*, 18 (1985) 503–518.
8 L. Ramaley, M.-A. Vaughan and W. D. Jamieson, *Anal. Chem.*, 57 (1985) 353–358.
9 H. Iwabuchi, A. Nakagawa and K.-I. Nakamura, *J. Chromatogr.*, 414 (1987) 139–148.
10 Y. Kushi and S. Handa, *J. Biochem.*, 98 (1985) 265–268.
11 C. J. Clemett, *Anal. Chem.*, 43 (1971) 490.
12 T. T. Chang, J. O. Lay and R. J. Francel, *Anal. Chem.*, 56 (1984) 109–111.
13 D. Dekker, *J. Chromatogr.*, 168 (1979) 508–511.
14 B. E. Maryanoff, S. O. Nortey, J. F. Gardocki, R. P. Shank and P. Dodgson, *J. Med. Chem.*, 30 (1987) 880–890.