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TRACE DETECTION OF MODIFIED DNA BASES VIA MOVING-BELT LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY USING ELECTROPHORIC DERIVATIZATION AND NEGATIVE CHEMICAL IONIZATION

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SUMMARY

   Electrophoric derivatives of 5-methylcytosine and 5-hydroxymethyluracil nucleobases are determined using high-performance liquid chromatography–mass spectrometry coupled via a moving-belt interface. Standards as well as samples derived from DNA are analysed. As little as 9.9 pg (signal-to-noise ratio 5) and 180 fg (signal-to-noise ratio 10) of the respective nucleobases are detected in the electron-capture negative chemical ionization mode, and linear responses are observed over a moderate dynamic range. In a comparison study, liquid chromatography–electron-capture negative chemical ionization mass spectrometry is found to have a sensitivity comparable to gas chromatography–electron-capture negative chemical ionization mass spectrometry for 5-hydroxymethyluracil. A detection limit of 60 fg (signal-to-noise ratio 5) by gas chromatography–mass spectrometry is only three-fold better than the amount detected by liquid chromatography–mass spectrometry using the same mass spectrometer.

INTRODUCTION

   The usefulness of mass spectrometry (MS) as a tool for structural elucidation of nucleic acid constituents has been well documented. Many modes of ionization have been employed including electron impact ionization[1,2], chemical ionization (CI)[3], fast atom bombardment[4,5], field desorption[6], and thermospray[7]. Some of these techniques, however, while providing important structural information, do not have the sensitivity necessary for the analysis of components derived from small amounts of human DNA.

   Recently we reported on the application of gas chromatography (GC)–elec-

tron-capture detection (ECD) and GC–electron-capture negative CI-MS (ECNCI-MS) for the analysis of modified bases of DNA using electrophoric chemical derivatives[8–11]. Picogram and sub-picogram amounts of 5-methylcytosine (5MC) and 5-hydroxymethyluracil (HMU), respectively, were detected. In this work we found that post-column derivatization cleanup of the sample by high-performance liquid chromatography (HPLC) was useful. This has led us to investigate the potential applicability of LC–ECNCI-MS to this problem. Not only would this potentially simplify the analysis, but it would eliminate the difficulty of concentrating picogram quantity samples down to the small volumes necessary for on-column injection onto a capillary GC column.

While there are a number of applications of LC–MS for quantitative analysis using a variety of interfaces[12–15], there is a paucity of work concerning the use of LC–MS for quantitative analysis at the subnanogram level[16,17], or describing the use of LC ECNCI-MS[18]. In this paper we report the results of such studies using a moving-belt interface equipped with a spray device recently developed in our laboratory[19].

The pentafluorobenzyl (PFB) derivatives of 5MC and HMU, which have good electron-capture properties, were examined, both as standards and derived from DNA. These electrophoric derivatives are well suited for work with the moving-belt LC–MS interface, being non-ionic and adequately volatile. Moreover, the HPLC mobile phases used for the separation of the analytes and the internal standards are compatible with the solvent requirements of the interface. Finally, our examination of the same samples by GC–ECNCI-MS provides a convenient and unique opportunity for a comparison of the GC–MS and LC–MS methods of analysis using the same mass spectrometer.

EXPERIMENTAL

Mass spectrometry

A Finnigan (San Jose, CA, U.S.A.) 4021B mass spectrometer equipped with a moving-belt HPLC–MS interface (Kapton® belts) and an HP-5890 gas chromatograph was used in all experiments.

The mass spectrometer was operated in the ECNCI mode (0.20 to 0.25 Torr methane) with a source temperature of 250°C. The reagent gas pressure was optimized during each experiment for maximum sensitivity. Quantitative data were acquired using selected ion monitoring (SIM), with dwell times of 0.5 s per selected unit mass. Full-scan spectra were recorded by scanning from 50–700 a.m.u. in 1 s.

The compounds were deposited on the belt by spraying the HPLC eluent via an electrically heated spray device[19]. To determine the transfer efficiency of the sprayer, we deposited a sample directly onto the belt with a hypodermic syringe, and compared the peak area with that obtained via flow injection of the same amount. The syringe was assumed to provide a 100% transfer of solute to the belt. The vaporizer temperature was set at 300°C for the HMU derivative and 385°C for the 5MC derivative. The cleanup heater of the interface was set at 330°C in both cases. At 300°C the belt speed was 3 cm/min, and at 380°C it was increased to 4 cm/min to avoid damage to the belt.

GC–MS analyses were carried out using an HP-5 cross-linked 5% phenyl-

methyl silicon capillary column, 25 m × 0.32 mm I.D., 0.25 μm film thickness. The column was interfaced directly to the mass spectrometer. Scan rates and programs are the same as those described for the LC–MS experiments. The oven was programmed from 60–300°C at 30°C/min. Helium was used as a carrier gas. The head pressure was maintained at 15 p.s.i. Injections of 1.5 μl or less were made in the on-column mode.

HPLC

LC was performed with a Waters (Milford, MA, U.S.A.) M 6000A solvent delivery system and a Rheodyne (Cotati, CA, U.S.A.) 7125 injection valve (20-μl loop). A Kratos (Ramsey, NJ, U.S.A.) Spectroflow 773 UV detector was used to optimize the separations and to determine the retention time of a non-retained compound, t_0.

The column was a Supelcosil LC-18-DB (150 × 4.6 mm, either 5- or 3-μm particles), protected by a Supelcosil LC-18-DB guard column (20 × 4.6 mm, 5-μm particles) from Supelco (Bellefonte, PA, U.S.A.). The flow-rate was maintained at 1 ml/min, and the elution volume of a non-retained compound (V_0) was determined to be 1.9 ml. In order to reduce adsorption of the derivatives, the columns were pretreated with a 10 mM acetic acid, adjusted to pH 4.5 with tributylamine and containing 100 mg/l each of adenine, guanosine, cytosine, thymine and 5MC.

Water and methanol were HPLC grade, purchased from Baker (Phillipsburg, NJ, U.S.A.).

All evaporations were carried out on a Speed Vap.

Adducts and derivatives

Internal standards were prepared by replacing one of the PFB groups with a tetrafluorobenzyl (TFB) group. The respective internal standards were added just prior to the final GC or HPLC analysis, and therefore do not serve as recovery standards. The TFB derivatives are only used to quantitate the final concentration of the analyte.

The work-up of calf thymus DNA for the analysis of HMU[17] was accomplished in the following manner. The DNA was hydrolyzed by heating an appropriate amount in 3 M hydrochloric acid for 5 h at 95°C. Upon completion, each sample was cooled to room temperature and evaporated. The sample was reconstituted in 500 μl of 10 mM potassium phosphate pH 4.6 and applied to silanized Pasteur pipettes packed with 500 mg of C_{18} silica (prewashed with 3 ml each of methanol and water). The sample was eluted with methanol using vacuum, and the eluent collected and evaporated. To each dried sample was added a stirring bar and 250 μl of a stirring potassium carbonate suspension (7.76 mg in 20 ml of acetonitrile). The sample was stirred at medium speed for 20 min, after which 250 μl of a stock PFB bromide solution (28 μmol in 20 ml of acetonitrile) was added, and the solution gently swirled for 1 h. The sample was then heated at 60°C with medium stirring for 3 h, cooled to room temperature and evaporated.

A stock solution of tetrabutylammonium sulphate (TBAS) was prepared (166.4 mg of TBAS in 20 ml of 1 M potassium hydroxide and 200 μl were added to each sample. After stirring at medium speed for 20 min at room temperature, 200 μl of a PFB bromide solution (700 μmol in 20 ml of dichloromethane) were added and stirring was continued for 6 h at room temperature. The sample was then washed with

500 μl of dichloromethane and water. The aqueous phase was removed and washed with an additional 500 μl of dichloromethane. The dichloromethane layer was then combined and evaporated. The sample was further purified by reversed-phase (C_{18}; acetonitrile–water, 70:30) and normal-phase (silica; isooctane–isopropanol, 99:1) HPLC respectively. Using retention data from standards, the appropriate 2-ml fraction was collected and evaporated. Prior to analysis each sample was reconstituted in warm toluene containing the internal standard. Additional details for the entire procedure are presented in ref. 17.

The work-up of calf thymus DNA for the analysis of 5MC[11] began with a hydrolysis of the DNA in 200 μl of formic acid at 150°C for 3 h. After evaporation, 100 μl of a PFB bromide solution (0.1 M in acetonitrile) and 1–2 mg of dry potassium carbonate was added. Each sample was heated at 60°C, with vortexing every 30 min. After 3 h, 500 μl of ethyl acetate–dichloromethane–acetonitrile (3:1:1) (solvent A) was added and the solution was applied to a silanized Pasteur pipette packed with 500 mg of end-capped propyl-cyano silica that had been prewashed with 2 ml of solvent A. The column was then washed with 2 ml of solvent A, and 4 × 500 μl of methanol. The final two methanol washes were collected and evaporated.

The dried residue was treated with 100 μl of pivalic anhydride (0.1 M) and 100 μl of dimethylaminopyridine (DMAP) (0.2 mM) in dry acetonitrile. After heating for 1 h at 60°C, 241 pg of the internal standard were added. The sample was diluted with 500 μl of acetonitrile–2-propanol (9:1) (solvent B) and applied to a silica extraction column. After washing with 2 ml of hexane–dichloromethane (3:1), the compounds were eluted with 4 × 500 μl of solvent B. The final two washes were collected and evaporated. The extract was further purified by reversed-phase HPLC (C_{18}, 20–70% acetonitrile in 10 min, then to 80% acetonitrile in 10 min) with blind collections of the appropriate 2 ml fractions. The samples were evaporated for storage. Prior to analysis the sample was reconstituted in warm toluene. Additional details for the entire procedure are presented in ref. 11.

Standard solutions of the 5MC derivative were prepared in concentrations ranging from 10 to 282.5 pg/μl, containing 39 pg/μl internal standard. The HMU derivatives were prepared from 0.190 to 11.9 pg/μl with 6.2 pg/μl internal standard. All weights refer to the base content of the derivatives. All standard solutions were made in methanol using acid-cleaned and gas-phase silanized glassware, pipettes, and syringes to avoid adsorption and cross-contamination. Injection volumes for HPLC were 3 μl for the standard solutions, and 10-20 μl for the DNA extracts.

RESULTS

This work focused on the analysis of two modified DNA bases, 5MC (**1**) and HMU (**2**). These compounds were converted to the corresponding pivalyl-N1-pentafluorobenzyl-5-methylcytosine, (**1a**) and 5-pentafluorobenzyloxymethyl-N1,N3-bis(pentafluorobenzyl)uracil (**2a**) derivatives. Internal standards were prepared by replacing one of the PFB groups in each of these compounds by a 2,3,5,6-tetrafluorobenzyl group. Thus the molecular weights of the analytes differ from their respective internal standards by 18 mass units, corresponding to the replacement of one fluorine by a hydrogen atom. All structures are shown in Fig. 1.

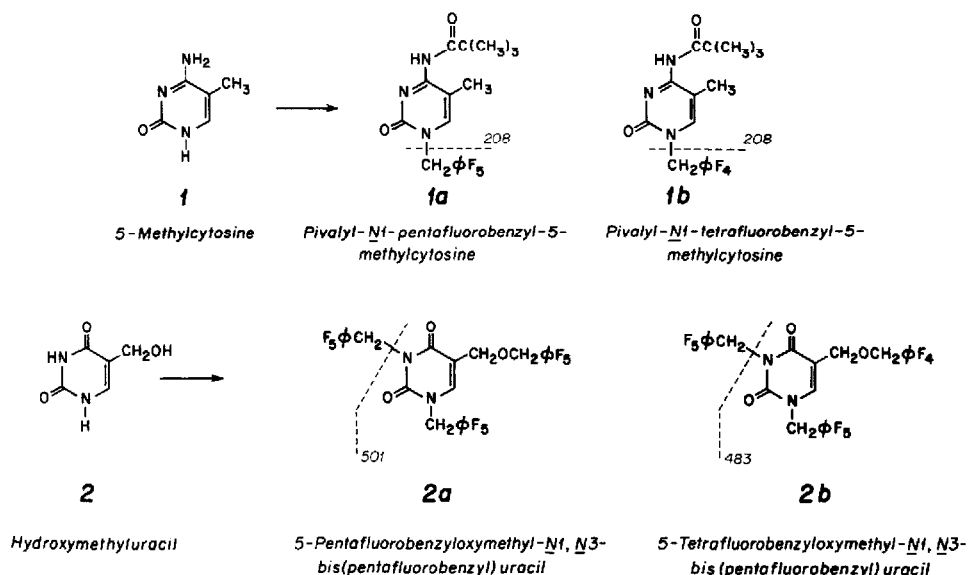Mass spectral characteristics of the derivatives under ECNCI-MS conditions

Fig. 1. Structures of 5-methylcytosine and 5-hydroxymethyluracil and their derivatives showing cleavages leading to diagnostic ions. Φ = Phenyl group.

were examined using the moving belt and by GC–MS. The spectra recorded off of the moving belt were identical with those recorded by GC–MS, and changes in the LC interface temperatures had no apparent effect on the fragmentation of the derivatives. No attempts were made to study the effect of lowering the ion source temperature, because at 250°C the spectra already show only one ion, and the higher temperature prolongs the operating lifetime of the source.

The derivatives fragment by loss of a fluorobenzyl group to yield essentially one ion. As shown in Fig. 1, this fragmentation leads to an ion of m/z 208 for both the 5MC derivative (**1a**) and its internal standard (**1b**). Chromatographic resolution of this pair of compounds is therefore necessary for LC–MS analysis using single ion monitoring. No such separation is necessary, however, for the analysis of the HMU derivative (**2a**) and its tetrafluorobenzyl internal standard (**2b**) since the fragmentation of these compounds leads to ions of different mass, m/z 501 and m/z 483, respectively.

As a first step in the development of an LC–MS assay for the compounds of interest we optimized the moving belt interface conditions. Subsequently, detection limits and calibration curves were obtained for the analytes using LC–MS. Finally, samples derived from DNA, which had been previously quantified by GC–ECD and GC–ECNCI-MS, were analyzed by LC–MS.

Better detection limits were obtained throughout this work for the HMU derivative as opposed to the 5MC derivative, consistent with the better GC behavior of the former compound observed in our laboratory.

Optimization of the HPLC–MS interface

Vaporizer temperature. An obvious requirement for LC–MS via a moving-belt interface is the ability to vaporize the analyte into the ion source. Consequently, the

determination of the optimum temperature for the flash vaporizer is an essential first step in this type of study. Accordingly, we measured the response of the detector to both model compounds by monitoring the single ion current of each derivative as a function of vaporizer temperature as they were spray-deposited onto the belt (Fig. 2). Significant differences are found in the behavior of the two compounds. Whereas the response of the 5MC derivatives **1a** increases in an exponential manner without reaching a maximum below 300°C, that of the HMU derivative **2a** rises very fast between 150 and 225°C, reaching a plateau above 250°C. We later tried a vaporizer temperature of 385°C for the 5MC derivative and observed a two-fold higher response than at 300°C. No experimental difficulties were encountered when the interface was kept at 385°C for several hours.
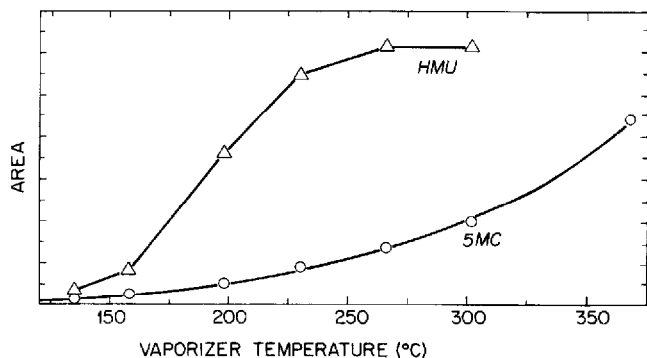

Fig. 2. Response of equimolar amounts of the 5MC and HMU derivatives *versus* vaporizer temperature. Technique: flow injection analysis with single ion monitoring; source temperature: 250°C; belt speed: 3 cm/min; methane CI pressure: 0.21 Torr.

Transfer efficiency. The transfer efficiency was established by injecting (rotary valve) 3-μl volumes of sample through a capillary tube connected to the spray device (flow-rate 1.0 ml/min), and comparing the response against that obtained by direct deposition of 3 μl of the same solution onto the belt with a syringe. The recovery was 74 ± 5% for 28 pg of 5MC and 72 ± 4% for 25 pg of HMU. It is notable that these recoveries are at the picogram level. Moreover, for the HMU derivative an essentially constant transfer efficiency is observed over three orders of magnitude in the range from 0.60 to 25 pg. These observations further support the utility of the moving belt interface for quantitative analysis.

HPLC–MS analysis

The 5MC derivative and its corresponding tetrafluorobenzyl internal standard both have the same diagnostic ion at m/z 208. Thus, in order to maintain reliable quantitation, it is necessary to achieve good chromatographic resolution of both compounds. Using methanol–water (90:10) as an eluent, the compounds are nearly baseline resolved with a capacity factor (k') of 0.4 for **1a**.

Since the HMU derivative and its internal standard, **2a** and **2b** respectively, have diagnostic ions with different masses, we had greater flexibility in developing the

chromatographic conditions. Nevertheless, as our long range goal is to develop a general method for the extraction, derivatization and analysis of as many modified bases as possible, we chose the same chromatographic conditions as those used for the analysis of the 5MC derivatives. The resolution of the HMU derivative and its internal standard was determined to be greater than 2.0 with a k' of 1.2 for **2a**. The separation of the four derivatives (two analytes and their corresponding internal standards) by LC–MS is shown in Fig. 3.
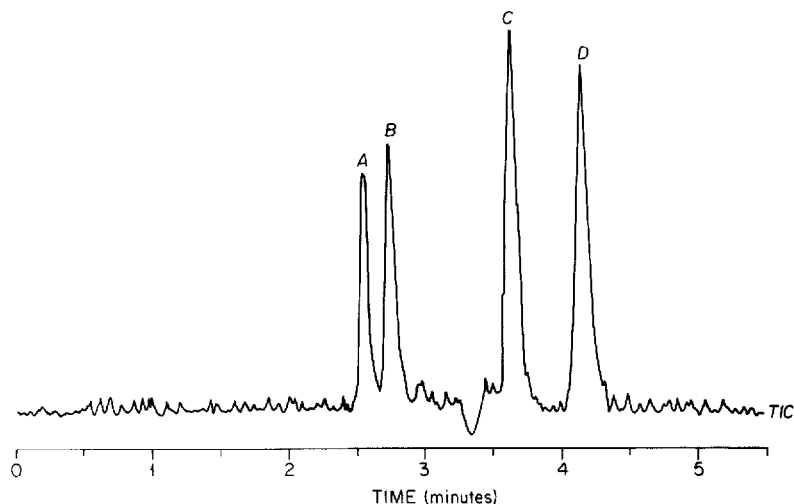

Fig. 3. Chromatogram of the 5MC and HMU derivatives and their respective internal standards. Column: Supelcosil LC-18-DB, 150 × 4.6 mm, 3 μm particles; eluent: methanol–water (90:10); flow-rate: 1.0 ml/min; vaporizer temperature: 300°C; belt speed: 3 cm/min; methane CI pressure: 0.21 Torr; ions monitored: m/z 208, 483 and 501. Peaks: A = 80 pg internal standard 5MC; B = 80 pg 5 MC; C = 2.25 pg internal standard HMU; D = 1.62 pg HMU. TIC = Total ion current.

Calibration curves for **1a** and **2a** were obtained by triplicate 3-μl injections at five different concentrations. For **1a** a linear response ($y = 0.002x + 0.037$) was obtained with a correlation coefficient $r^2 = 0.9921$ in the range corresponding to 10 to 280 pg of the base. Similarly, a linearity over the range from 0.56 to 36 pg of the free base with a value of $r^2 = 0.9978$ was obtained for the calibration curve of **2a** ($y = 0.923x + 0.060$). Response factors on a weight basis were determined to be 0.822 (S.D. = 4.6%) for 5MC and 1.04 (S.D. = 4.5%) for HMU.

The generally favorable behavior of these analytes encouraged us to establish the detection limits for these compounds by LC–MS. Chromatographic profiles of derivatized 5MC and HMU at or near the detection limits are shown in Fig. 4. Detection limits of 9.9 pg for 5MC (signal-to-noise ratio 5) and 180 fg for HMU (signal-to-noise ratio 10) are observed. These values significantly advance the sensitivity that has been achieved to date by LC–MS irrespective of the nature of the interface. Precision and accuracy data for concentrations within the working range of both analyses are shown in Table I.
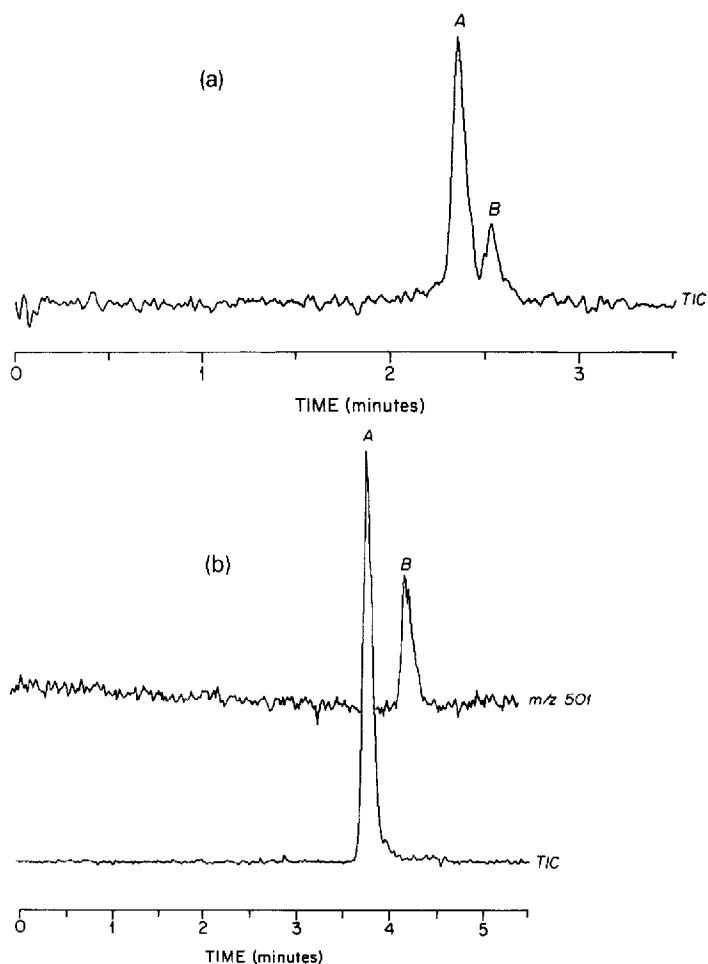
Fig. 4. (a) Demonstration of the detection limit for Piv-PFB-5-MC. Column: Supelcosil LC-18-DB, 150 × 4.6 mm, 3-μm particles; eluent: methanol–water (90:10); flow-rate: 1.0 ml/min; vaporizer temperature: 385°C; belt speed: 4 cm/min; methane CI pressure: 0.21 Torr; ion monitored: m/z 208. Peaks: A = 118.3 pg internal standard; B = 9.9 pg 5MC. Demonstration of the detection limit for (PFB)$_3$HMU. Column, eluent and flow-rate as in (a); vaporizer temperature: 300°C; belt speed: 3 cm/min; methane CI pressure: 0.21 Torr; ions monitored: m/z 501 and 483. Peaks: A = 6.0 pg internal standard; B = 185 fg HMU.

TABLE I

ACCURACY AND PRECISION DATA FOR LC–MS ANALYSIS

Compound	Amount injected (pg)	Amount measured (pg)	Relative error (%)	Coefficient of variation (%)
5MC	28.0 (n = 4)	24.6 ± 3.5	− 12.0	9.3
	55.0 (n = 6)	54.6 ± 5.7	− 0.7	6.7
	282.8 (n = 6)	282.8 ± 14.2	+ 0.1	2.4
HMU	1.9 (n = 6)	1.7 ± 0.3	− 8.9	11.9
	10.9 (n = 6)	11.6 ± 0.5	+ 6.4	3.5
	32.1 (n = 6)	32.4 ± 0.7	+ 0.9	4.7

Analysis of DNA extracts

Samples of DNA, previously analyzed by GC–ECD and GC–ECNCI-MS[11,20], were also examined. The LC–MS chromatogram of the 5MC derivative and its internal standard isolated from calf thymus DNA is shown in Fig. 5a. The data correspond to 410 pg of 5MC in 100 ng DNA, equivalent to a mole percentage of 1.0 for 5MC relative to the total base content of the DNA sample. The latter was determined by independent acid hydrolysis of the DNA to the bases and then subsequent quantitation of the bases by HPLC with UV detection[11]. This result compares favorably with a value of 1.2 ± 0.1 mole% for the same analysis by GC–ECD[11], and the 1.2
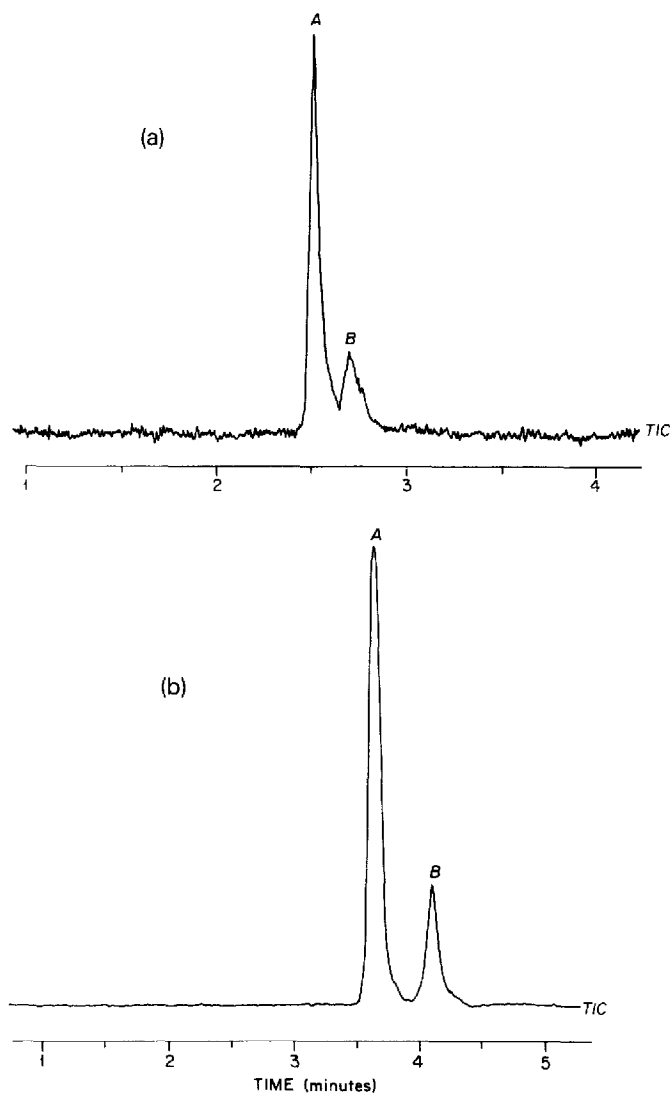

Fig. 5. (a) Determination of 5MC in 100 ng of calf thymus DNA. Conditions as in Fig. 4a. Peaks: A = 96.4 pg in internal standard; B = 5MC. (b) Determination of HMU in 7.8 mg of calf thymus DNA. Conditions as in Fig. 4b. Peaks: A = 33.2 pg internal standard; B = HMU.

mole% found by Crain and McCloskey[21] using isotope dilution MS. Similarly, LC–MS analysis of a sample of human leucocyte DNA found 0.63 mole% of 5MC compared to 0.8 ± 0.04% by GC–ECD.

An HMU sample derived from 7.8 mg of calf thymus DNA was also analyzed by LC–MS. A representative chromatogram is shown in Fig. 5b. The data correspond to 0.7 ng of HMU in the hydrolyzed DNA sample. This is in good agreement with values of 0.7 ng by GC–ECNCI-MS and 1.0 ng by GC–ECD[20] for this hydrolyzed DNA preparation. Similarly, 4.4 pg of HMU base was measured in the sample obtained from 7.8 µg of DNA compared to 3.0 pg by GC–ECD.

This value is artifactual since acid hydrolysis of thymidine, using the conditions described here, yields thymine contaminated with 0.001% HMU. The problem can be corrected by purifying the HMU deoxynucleotide derived from DNA by HPLC prior to acid hydrolysis. The discrepancy in the values of HMU found in the smaller *vs.* the larger DNA extracts can be attributed to the higher susceptibility of the smaller sample to undergo oxidation. It is not associated with the LC–MS instrumental method, which has been demonstrated to be highly reproducible, and therefore, does not detract from the validity of the LC–MS method to detect picogram quantities of damaged DNA bases.

DISCUSSION

The results presented here provide convincing evidence regarding the capability to conduct quantitative analysis at the trace level by LC–MS via the moving-belt interface. This is, in part, facilitated by the versatility of the interface which allows selection of the optimal ionization operating conditions of the mass spectrometer for a given type of analyte, in this case ECNCI-MS.

A comparison of Figs. 4 and 5 shows that the sensitivity achieved by the method for pure standards translates well to the analysis of complex matrices. The 5MC peak in the chromatogram of the DNA hydrolysate (Fig. 5a) represents 25.3 pg. The peak height and signal-to-noise ratio of this peak are consistent with the 5MC peak in Fig. 4b resulting from the injection of 9.9 pg of pure standard.

An examination of the HMU internal standard peaks in Figs. 4a and 5b again shows good correlation in terms of the signal-to-noise ratios for the standard and the sample. It is noteworthy that the HMU peak in the hydrolysate chromatogram (Fig. 5b) corresponds to 20 pg detected, and that this is 100 × the amount shown for the pure standard in Fig. 4a. The HMU peak in Fig. 4a is barely visible next to the internal standard in the bottom trace, which represents the TIC (m/z 501 + m/z 483).

In this work, we observed better performance for HMU compared to 5MC in two general respects. First, initial attempts to handle solutions containing trace amounts of these compounds in non-silanized glassware resulted in erratic results with 5MC while consistent measurements were obtained for HMU. No memory effects were observed, however, on the moving belt for either compound when operated at a constant temperature.

Secondly, under LC–MS conditions the response of the 5MC derivative dropped off precipitously as the water content of the mobile phase was increased. For example, at 70% aqueous methanol, a 5% increase in the water concentration resulted in a 50% decrease in the measurable peak area. The mobile phase used for the

LC–MS analysis of the 5MC derivative (methanol-water, 90:10) represents a compromise between minimizing this problem and selecting an optimum composition for the LC separation. This phenomenon was not observed under LC–UV conditions, nor was it encountered in the development of the HMU assay.

For both the 5MC and HMU derivatives we observed, using MS detection, that there was no difference, within experimental error, in the recovery of the analyte for manual spotting onto the belt *vs.* spray deposition after flow injection. On the other hand, under identical solvent composition and flow conditions, when spray deposition followed HPLC a significantly lower response was observed. This difference amounted to 40% less recovery for the 5MC derivative and 75% less recovery for the HMU derivative.

Related to these problems, and perhaps to other aspects of the system as well, the relative molar response of the HMU derivative to the 5MC derivative by LC-MS was about 100: 1 under the conditions selected for most of the work (see Fig. 3). This is in contrast to a more comparable molar response of the two derivatives (3: 1) under GC- MS conditions. Thus 180 fg of HMU could be detected by LC-MS compared to 10 pg of 5MC.

The better performance of the HMU derivative over the 5MC derivative in these respects is probably due, in part, to the active hydrogen on the latter compound, resulting in greater surface interaction. This points to the importance of optimizing the structure of the derivative in order to take full advantage of LC-MS for high sensitivity.

The HMU analysis of calf thymus DNA affords a rather unique opportunity to compare LC–ECNCI-MS to the vapor phase methods of GC–ECD and GC–ECN-CI-MS. The results are presented in Table II. Rather striking is the good correlation of the results and, in particular, the comparable detection limits (180 fg and 60 fg) attainable by LC-ECNCI-MS and CG-ECNCI-MS, respectively. This comparison of course does not take into account the differences in band widths associated with LC and GC. It is conceivable that the correspondence in the detection limits would be even closer if peaks of identical width could be attained. Clearly, such comparisons are also compound dependent. For example, it is unlikely that substances that are highly volatile or highly ionic or very polar in nature will behave in an identical

TABLE II

COMPARISON OF HPLC-MS AND GC–ECD RESULTS FOR HMU

Sample	*Method*	*Amount*[a]
7.8 mg Calf thymus DNA	GC − ECD	1.0 ng / 7.8 mg
	GC − ECNCI-MS	0.7 ng / 7.8 mg
	LC − ECNCI-MS	0.7 ng / 7.8 mg
7.8 μg Calf thymus DNA	GC − ECD	3.0 pg / 7.8 μg
	LC − ECNCI-MS	4.4 pg / 7.x μg

Detection limits: GC − ECNCI-MS: 60 fg (signal-to-noise ratio 5)
LC- ECNCI-MS: 180 fg (signal-to-noise ratio 10)

[a] As explained in the text, the amount of HMU is arbitrary since acid hydrolysis converts 0.001% of thymidine to HMU.

manner by LC-MS and GC–MS. However, these results indicate that, given suitable physical characteristics of an analyte, LC–MS via the moving-belt interface can compare favorably with CC-MS as a quantitative tool for trace analysis.

In summary, we have observed that LC-MS with a moving-belt interface can be nearly as sensitive as GC–ECNCI-MS for quantifying a derivatized DNA base as long as an appropriate derivative is selected. Furthermore, electrophore-based analytical methodology terminating in LC–MS shows great promise for the sensitive determination of alkyl and related chemical damage to DNA.

ACKNOWLEDGEMENTS

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