ELSEVIER

Journal of Chromatography A, 794 (1998) 97–108

JOURNAL OF CHROMATOGRAPHY A

Review

Analysis of DNA adducts by capillary methods coupled to mass spectrometry: a perspective

W.A. Apruzzese, Paul Vouros*

Department of Chemistry and Barnett Institute, Northeastern University, Boston, MA 02115, USA

Abstract

The analysis of DNA adducts, important molecular biomarkers indicative of potential cellular damage by covalent attachment to DNA, is discussed. The paper focusses on a discussion of the current status in the analysis of DNA adducts via the use of capillary high-performance liquid chromatographic and capillary electrophoretic methods coupled to tandem mass spectrometry. © 1998 Elsevier Science B.V.

Keywords: Reviews; Mass spectrometry; DNA adducts

Contents

1. Introduction

Retrospective epidemiological studies indicate that various chemicals are often implicated in the etiology of human cancer. These chemicals may be formed naturally or they may be present in the environment, in our food or the water we drink. The identification and structural characterization of these unknown chemical factors is a major goal of cancer prevention. DNA adducts, i.e., covalent modifications of DNA by a chemical or its metabolites, are important to the carcinogenic process and to disease states. For example, recent studies involving breast cancer give evidence for a greater than 5-fold increase in the number of adducts present in the cancerous as compared to the normal breast tissue [1]. Risk assessment can utilize information about important xenobiotic chemical constituents whose effects are mediated through DNA adducts. However, knowledge about both the quantities and the identities of the DNA adducts is required to enable proper assessment of their effects. It is known that certain

*Corresponding author.

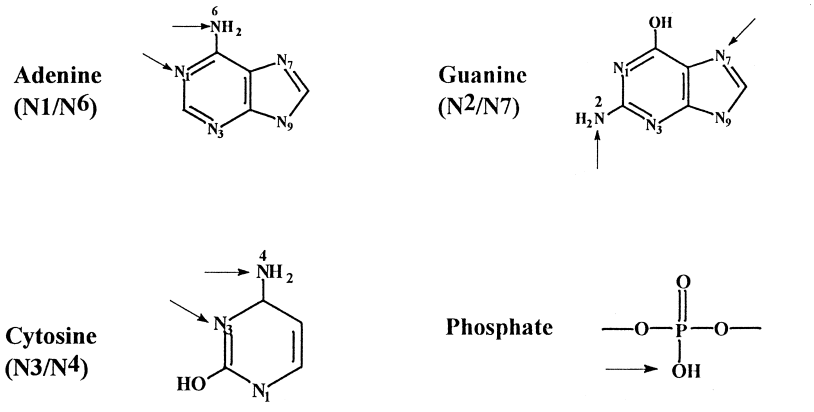
0021-9673/98/$19.00
PII S0021-9673(97)00820-0

chemical carcinogens display greater genotoxicity, or persist longer in the cell, when covalently bound in specific configurations. Therefore, even if these adducts are formed at lower levels than the other possible isomers, their detection and structural characterization is critical for any cancer risk assessment. Only then can this knowledge be used in fields such as human health or ecological system health assessment. For exposure assessment, the detection and quantitation of all adducts, irrespective of their relative genotoxicity is important.

Adducts may be formed by the covalent bonding of xenobiotic (or even endogenous) substances with DNA. Activation (enzymatic or other) of these agents may lead to their covalent attachment to DNA. Each of these agents may bind to specific sites on the nucleotide base or even the sugar phosphate as shown in Fig. 1 [2]. Identification of the location of the adduct on the nucleic acid and for that matter, the location of the modified nucleobase in the DNA sequence, may help determine which mechanisms of cellular functioning may be affected. When the adduct binds at a site which is involved in hydrogen bonding with another base, it causes the strand to bulge. This kind of malformation in the strand may not allow for proper folding of the DNA, in which case the cell may not have access to all of the DNA sequences. Such a modification may also cause problems with the transcription process directly, by binding in the initiator sequence for that protein, or by not allowing the RNA polymerase to read the entire sequence by physically hindering the motion of the enzyme down the DNA chain.

In view of the above considerations, the detection and characterization of DNA adducts is of paramount importance. However, the analytical challenge associated with this task is considerable. The following numbers can help put the whole problem into perspective. If the analytical mass detection limits are, e.g. 10 pg, and we are interested in detecting one modification in 10^8 normal bases, this would translate into a requirement of ca. 1 g of tissue sample (typically 1 g of tissue may contain 1 mg of DNA). Since it is desirable to have about a 10-fold excess of analyte, this leaves two alternatives: either sample 10 g of tissue or improve the detectability by a factor of 10 or more. In line with such requirements, it is natural that radiolabeling methods have been playing a significant role in the effort to analyze DNA adducts.

Currently, ^{32}P post-labeling is the most widely accepted method for detection of DNA adducts. This method allows detection of as little as one adduct per 10^9–10^{11} normal nucleotides [3,4]. However, while the ^{32}P method is very sensitive, it lacks the ability to provide any structural information which is essential in order to fully understand how the adduct contributes to carcinogenesis. It is in this context that mass spectrometry can fill a major void. Research designed to identify unknown adducts or to provide



Potential sites of adduction of DNA bases by epoxide PAHs (National Research Council, 1989)

Fig. 1. Structures showing potential sites of adduction of DNA bases by PAH epoxides.

convincing confirmatory evidence about known adducts, must utilize techniques that offer powerful identification ability, assurance that the adducts are bound to DNA (not to RNA), and excellent limits of detection. Mass spectrometry (MS) meets many of these requirements. When liquid chromatography (LC) or capillary electrophoresis (CE) are used in conjunction with MS, it is possible to detect adducts, as well as to generate a wealth of structural information.

This review addresses some of the recent applications in capillary chromatographic and electrophoretic methods in combination with MS for the analysis of DNA adducts. It is not intended to be a comprehensive literature review but rather to convey the strategies associated with the detection of molecular biomarkers indicative of risk to cancer exposure. A review by Farmer and Sweetman provides a more general perspective on the detection of DNA adducts by mass spectrometry [5].

2. Sample preparation

The general strategy for recovery of DNA adducts involves the breakdown of the DNA polymer to produce a mixture of normal and modified deoxynucleosides (or deoxynucleotides) as outlined in Fig. 2. The goal then is to 'fish' out of this complex mixture the modified analogs which are present at trace levels. Thus, before proceeding with LC–MS analysis, in vitro or in vivo DNA samples have to be properly 'cleaned' in order to increase the column lifetime and to obtain viable results. Recovery of a reconstituted sample enriched in adducts is the goal. As columns continue to shrink in size (e.g., capillary), the cleanup requirements become more stringent. Separation of the adducts from the bulk of normal deoxynucleotides is generally accomplished by chromatographic methods by taking advantage of the differences in hydrophobicity between the species. Cleanup procedures may differ in their detail according to the specific adducts under consideration. See, for example, Refs. [6–8] which discuss the recoveries of three adducts —ethylated deoxyguanosine, the benzo[*a*]pyrene diol epoxide adduct of deoxyguanosine monophosphate and 1-N^2-propanodeoxyguanosine— which possess widely different hydrophobic properties.

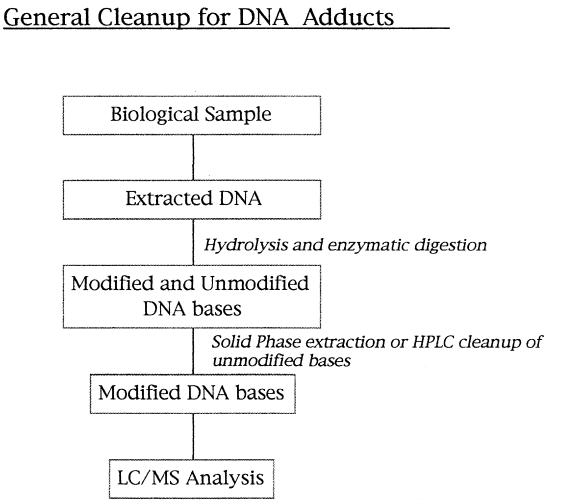


Fig. 2. Schematic of DNA sample preparation and general strategy for isolation of DNA adducts.

3. Mass spectrometric behavior of DNA adducts

In assessing the role of LC–MS in the analysis of DNA adducts, consideration should be given to the MS behavior of the compounds in conjunction with the modern techniques of ionization (i.e., thermospray (TS), continuous flow fast atom bombardment (CFFAB), electrospray ionization (ESI)) which are compatible with LC–MS coupling. The positive ion full scan TS, FAB, and ESI mass spectra of three different deoxynucleotide (-side) adducts are shown in Fig. 3 [9], Fig. 4 [10], and Fig. 5 [12].

The TS spectrum of the mitomycin C deoxyguanosine adduct (Fig. 3) typifies the behavior of these adducts under positive ionization. In addition to the protonated molecule, (M+H) m/z 570, important fragment ions are found at m/z 454, 244, 229, and 152. These masses correspond to specific blocks of the molecule and provide information as to the mass of the purine base and the bound carcinogen. Of particular significance is the observed cleavage of the glycosidic bond to give the ion of m/z 454 via the loss of the deoxyribose (116 Da) [9]. As seen from Figs. 4 and 5, many deoxyguanosine as

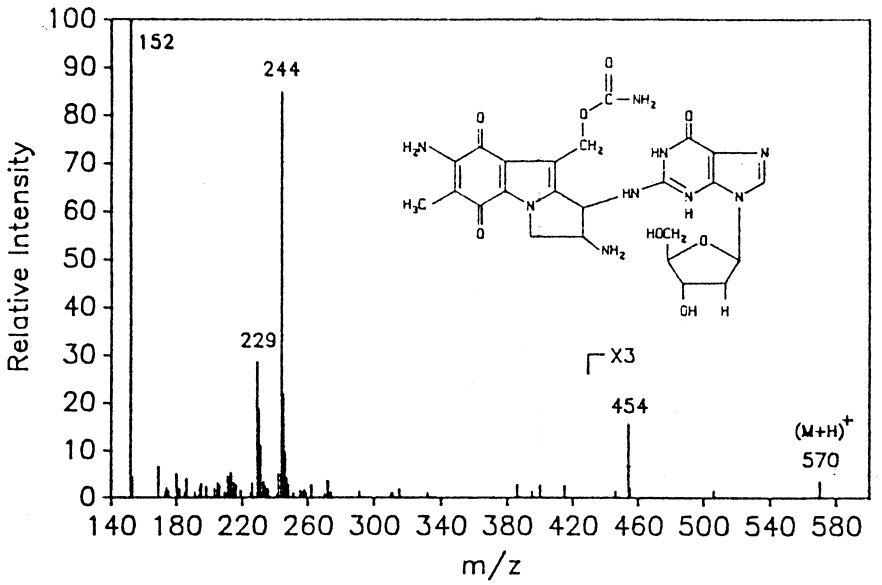


Fig. 3. Positive ion thermospray mass spectrum of deoxyguanosine adduct of mitomycin C. Reprinted from Ref. [9] with permission.

well as deoxyadenosine adducts exhibit the latter behavior. Consider for example, the FAB spectrum of the C8 adduct of N-acetylaminofluorene deoxyguanosine (C8-AAF-dG) [10]. In addition to the protonated molecule at *m/z* 489, a major fragment at *m/z* 373 reflects the loss of the sugar moiety. In fact, it should be noted that, under positive FAB ionization, the protonated molecule of the deoxynucleo*tide* analog of the same adduct also undergoes the same fragmentation to give the protonated base ion of *m/z* 373 [11]. In ESI, the cleavage of the glycosidic bond can be further controlled by manipulation of the skimmer cone voltage as illustrated in Fig. 5 which shows the mass spectrum of the heterocyclic aromatic amine adduct, C8-PhIP-dG [12]. Depending on the requirements, the abundance of either ion can be optimized for further analysis by tandem MS. As explained later, the above common spectral features are of special significance as they essentially define the mode of selective detection of DNA adducts in complex mixtures by LC–MS/MS.

4. Detection and characterization of DNA adducts by LC–MS/MS

Perhaps one of the classic examples that demonstrated the power of LC–MS for detecting modified nucleosides was presented by Edmonds et al. [13]. Analysis of the nucleosides from a digest of unfractionated *H. volcanii* tRNA by LC–UV produced the chromatogram shown in Fig. 6. A coelution of two isomeric monomethylated adducts, m^1G and Gm, at approximately 18 min is indicated in the UV chromatogram (Fig. 6a). Analysis of the mixture by thermospray LC–MS and plot of the mass chromatograms (Fig. 6b), shows a peak corresponding to the protonated molecules of the two isomers (*m/z* 298) at the same retention time. However, the two isomers can be independently resolved from the ion current profiles of their protonated base ions, *m/z* 152 for 1-methyl guanine and *m/z* 166 for guanine whose maxima, on close examination, are offset by 12 s. The isomer labeled as Gm presumably represents methylation on the ribose moiety. A dimethylated guanosine is also observed with its protonated molecule at *m/z* 312 along with its corresponding base ion at *m/z* 180. Full scan mass spectra in the above study were generated from as little as 10–50 ng of nucleosides.

Traditionally, the monitoring method of choice to improve detectability of an analyte in GC–MS (or LC–MS), has been selected ion monitoring (SIM). In the absence of co-eluting isobaric matrix interferences, a 10- to 100-fold enhancement in sensitivity over full scan spectral acquisition may be achieved

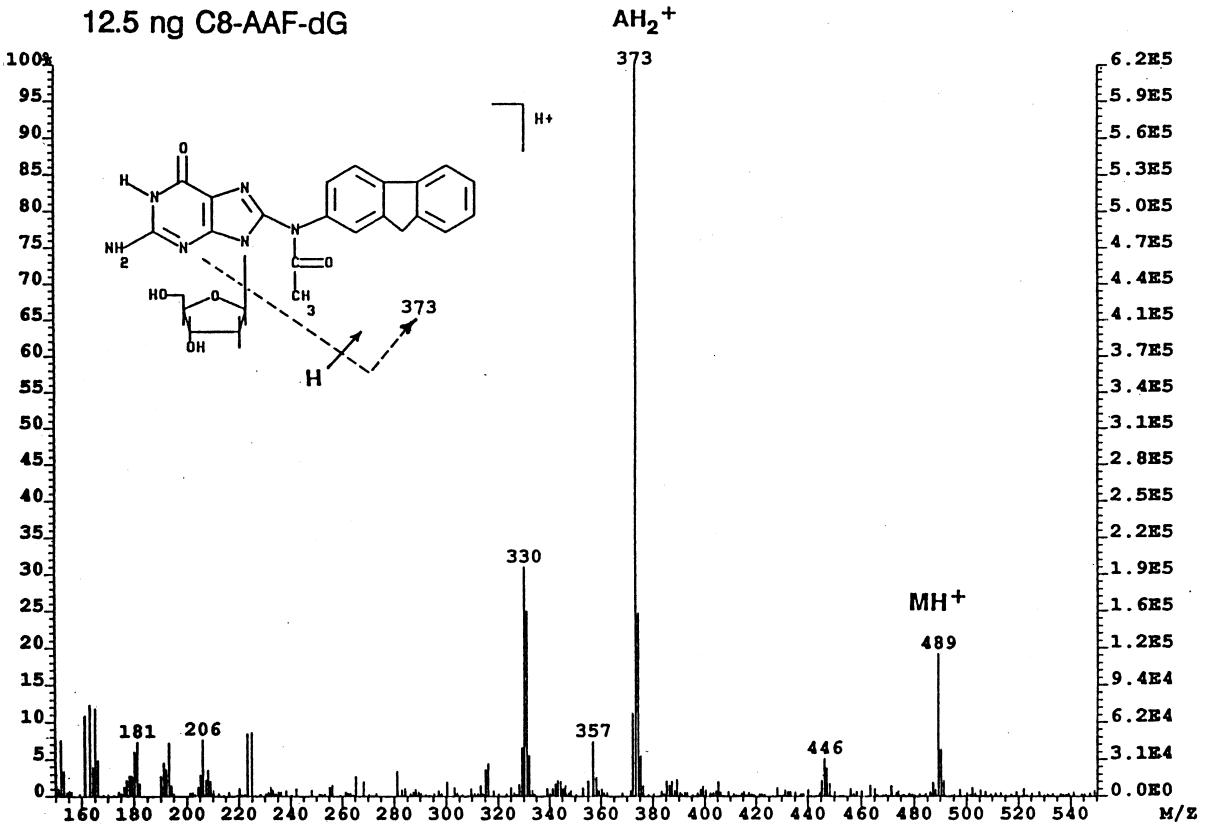


Fig. 4. Positive ion fast atom bombardment spectrum of C_8-deoxyguanosine adduct of N-acetylaminofluorene (C8-AAF-dG). Reprinted from Ref. [10] with permission.

by SIM. For example, a detection limit of 50 fmol (ca. 9 pg) for N^2,3-ethenoguanine was reported using LC–ES/SIM/MS [14]. On the other hand, tandem MS and, in particular, the triple quadrupole system, provides additional selectivity when used in the single (or multiple) reaction monitoring mode (SRM or MRM). Any ion losses in the tandem system, are more than compensated by the added degree of selectivity afforded by tandem MS. Accordingly, mass detection limits in the range of 30–50 fmol (10–30 pg) for a variety of adducts are typical by electrospray using the SRM scan mode. Thus, the latter approach appears to be the generally preferred mode of detection [11,12,15]. The fragmentation associated with the cleavage of the glycosidic bond to produce an aglycone ion (e.g., m/z 489→m/z 373 in Fig. 4) has been normally used for SRM detection.

In the analysis of unknown mixtures, it is convenient to initially screen for deoxynucleoside DNA adducts by scanning the tandem MS system in the constant neutral loss (CNL) mode (loss of the deoxyribose group, 116 Da). While the sensitivity is reduced to levels typical of those obtained in normal full scan mass spectral acquisition, detection by CNL simplifies the chromatogram as the only signals generated are from parents which fragment by loss of 116 Da, presumably deoxyribose-containing ions. In one of the earlier applications of this principle, analysis of the products formed in the in vitro reaction of AAAF with calf thymus DNA revealed the presence of deoxyadenosine adducts in addition to the expected deoxyguanosine analogs [11]. The same paper outlines in detail the benefits derived from the different scan modes of the triple quadrupole system and their role in the strategy for the analysis of DNA adducts. Fig. 7 [15] shows an example of the application of the CNL principle to the analysis of the hydrolysate of calf thymus DNA

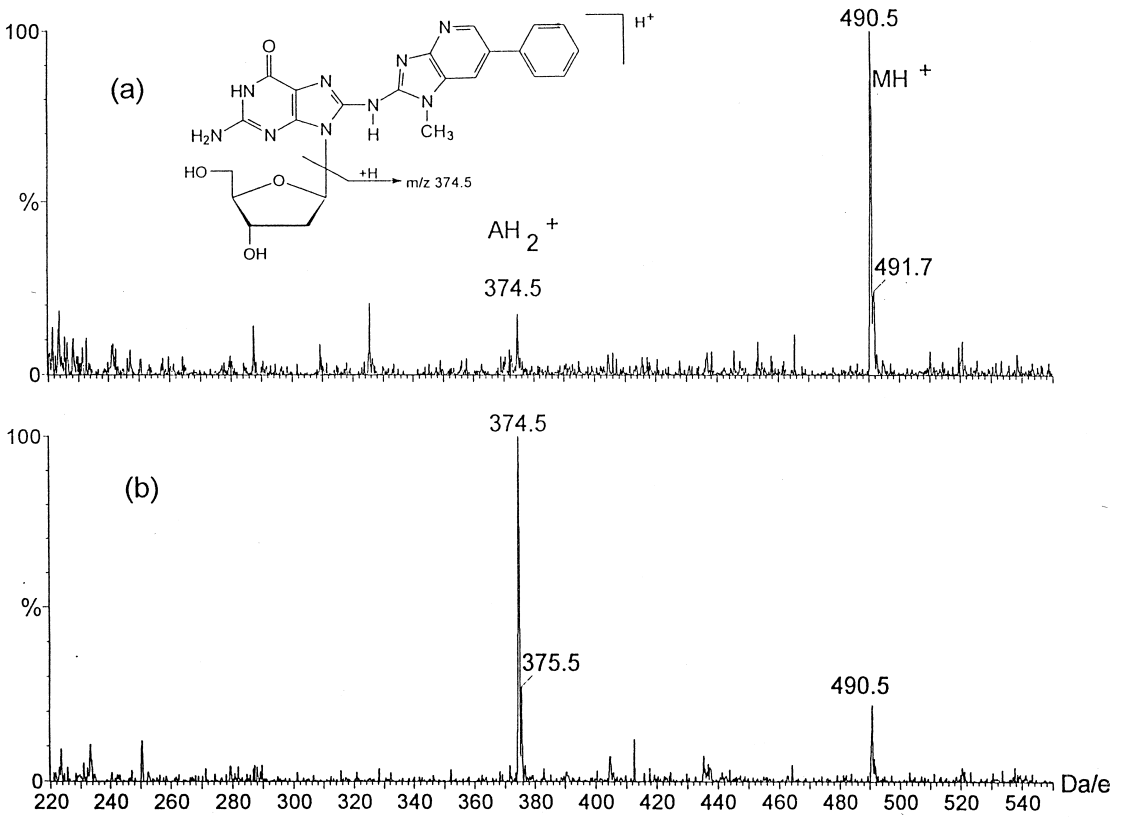


Fig. 5. Electrospray mass spectrum of dG-C8-PhIP obtained from 1.1 ng of standard introduced by capillary HPLC. (a) Focus lens=27 V; skimmer lens=40 V. (b) Focus lens=42 V; skimmer lens=53 V. Reprinted from Ref. [12] with permission.

modified by malondialdehyde (MDA). The protonated form of the M1-dG-dR adduct (m/z 304) shows up at 11:24 min in the mass chromatogram. Normal nucleosides not removed in the sample cleanup also fragment via the loss of the deoxyribose and, thus, their parent mass profiles appear in the chromatogram as well.

Following identification of the molecular masses of potential adducts, in a subsequent LC–MS analysis, mass spectra of the protonated molecules can be generated by CID to provide more detailed structural information. A frequently encountered problem in DNA adduct analysis from in vitro or in vivo mixtures is the occurrence of isomeric adducts. While such isomers may be differentiated from their CID spectra, some form of separation is essential in order to obtain an unadulterated fingerprint. It has been long recognized that CID spectra can provide a wealth of information about the structure of DNA adducts [16,17].

Of particular significance in that respect is the aglycone fragment ion which can be used to distinguish between isomeric DNA adducts. An example of this is given in Fig. 8 which compares the CID spectra C8-AAF-dG and N^2-AAF-dG [11]. The differences in their fragmentation patterns are rationalized in the figure. This example illustrates the power of collision mass spectrometry and its potential for DNA adduct characterization, especially when used in combination with HPLC as was the case in the example. On the other hand, the unequivocal characterization of totally unknown adducts from the CID spectra alone is not necessarily a trivial effort. The spectra are quite complex and relating spectral patterns to structure will require a more thorough understanding of the criteria which

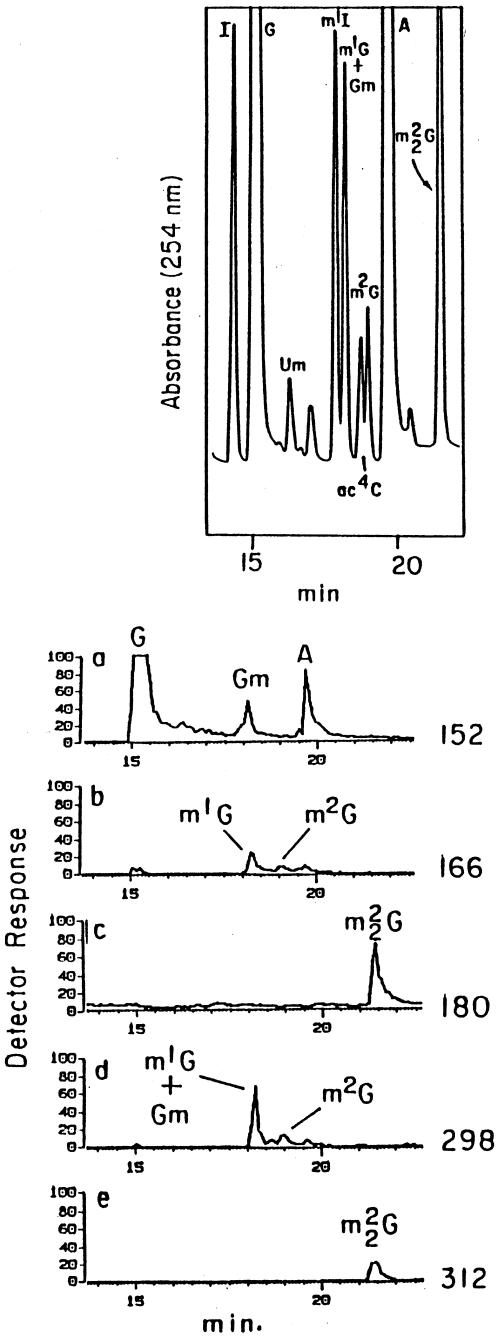


Fig. 6. (top) LC–UV analysis of nucleosides from 0.6 A_{260} units of unfractionated *H. volcanii* tRNA; detection at 254 nm. (bottom) Mass chromatograms of the same mixture analyzed by LC–thermospray-MS; panels (a), (b) and (c) represent profiles of protonated base ions and (d) and (e) profiles of MH^+ ions. Reprinted from Ref. [13] with permission.

govern the fragmentation of these molecules. Efforts to develop a more comprehensive database in this regard are timely and worthwhile especially since, according to at least our experience, low energy CID spectral patterns are very reproducible. As a result, even quantitative differences in ion peak intensities may be meaningfully related to molecular structures. It should also be noted that data from fundamental studies of the MS fragmentations of nucleosides, especially those conducted under positive chemical ionization conditions which result in even electron ions, are quite useful in the interpretation of CID fragmentations from (+)ES ionization [18].

5. Analysis of DNA adducts by capillary electrophoresis (CE)–MS

The growing interest in the coupling of capillary electroseparation methods to MS has recently led to the exploration of the usefulness of these techniques to the analysis of DNA adducts. One can point to several potential advantages of capillary zone electrophoresis (CZE) over capillary LC. For example, CZE is less expensive than HPLC (e.g., there is no need for costly high-pressure pumps or expensive columns), it is more efficient (theoretically an efficiency of 1 000 000 plates may be achieved), analysis times are more rapid in CZE and there is little time wasted between runs while waiting for column re-equilibration. In addition, CZE may be used in a diagnostic fashion to relate migration times to the pK_a values of suspect adducts. Furthermore, because of its low sample consumption, CZE can be used for rapid screening purposes to monitor for adduct formation, as pointed out by Jackim and Norwood [19].

In one of the earliest examples, CZE was used in combination with continuous-flow FAB for the analysis of a mixture of several deoxynucleoside adducts of different PAHs [20]. However, despite the demonstrated low mass-detection limits, an immediately recognized limitation of the technique was the poor concentration-detection limits. To place the issue in perspective, consider the case where 100 pg of adduct are reconstituted into a volume of 10 μl. In a conventional injection in CZE a ca. 5–10-nl volume of sample may be introduced into the capillary,

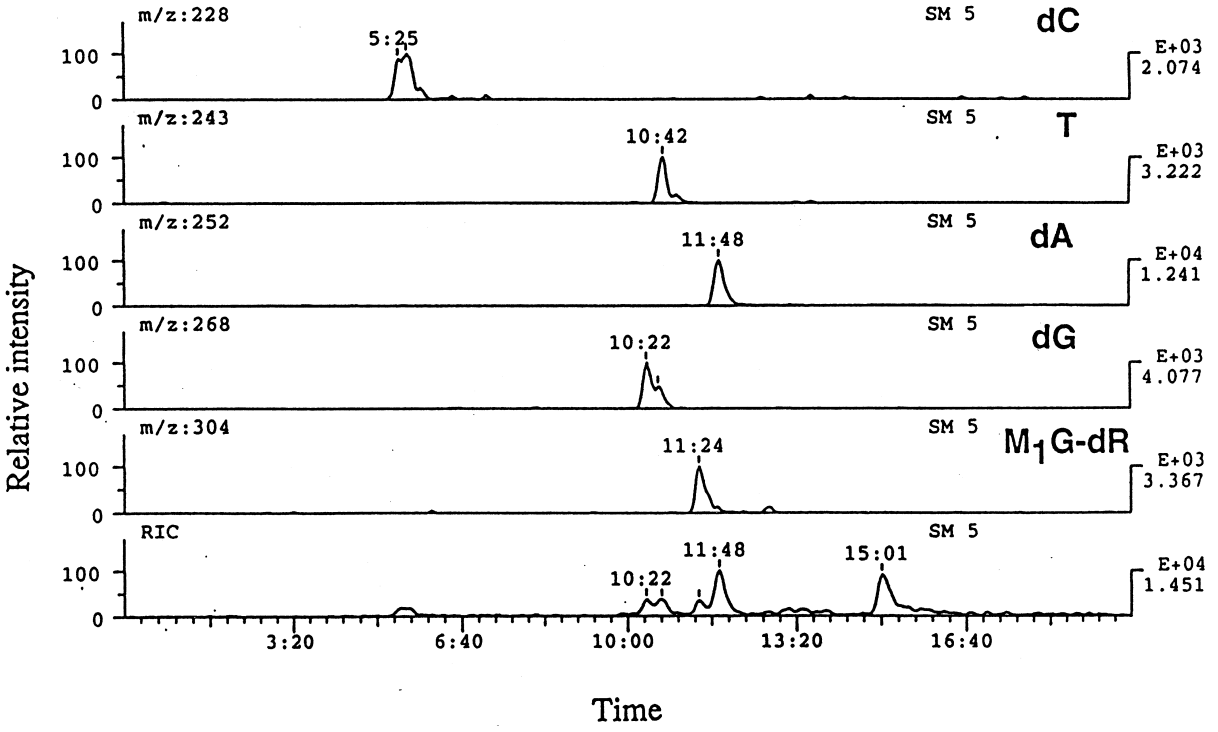


Fig. 7. LC–ESI-CNL-MS/MS analysis of the hydrolysate from NaMDA-modified calf thymus DNA. Chromatographic peaks at 5′25″, 10′22″, 10′42″ and 11′48″ in the RIC represent unmodified 2′-deoxynucleosides dC, dG, T and dA, respectively. The shoulder at 11′42″ was from M1G-dR. The reconstructed selected ion chromatograms for *m/z* 228, 243, 252, 268 and 304 correspond to MH^+ ions of dC, T, dA, dG and M1G-dR, respectively. Reprinted from Ref. [15] with permission.

corresponding to ca. 0.05–0.1 pg, a quantity well below the aforementioned mass-detection limits of 10–20 pg. The sample stacking technique originally proposed by Burgi and Chien [21], has been effectively utilized to overcome the drawback posed by the low injection volumes normally associated with CZE. In sample stacking the analyte is dissolved in a solution of low ionic strength and a substantial volume of the CZE capillary is filled with the analyte solution while the remaining capillary column space is occupied by the CZE buffer. With the field reversed, the electroosmotic flow displaces the dilute analyte-containing solution with the buffer, while the analyte ions ‘stack up’ against the boundary defined by the buffer and analyte solutions. The process is halted when an increase in the ion current indicates that virtually the entire capillary is occupied by the buffer solution. In this manner, a plug of analyte is effectively concentrated at the top of the CZE capillary and can then be subjected to normal analysis by reversing the voltage back to the normal operating conditions of capillary electrophoresis. The entire sequence of events is pictorially summarized in Fig. 9 [21] and can be completed in a few minutes.

Because of the nature of the stacking process, the method lends itself particularly well to the analysis of negatively charged species and, as a consequence, to the analysis of nucleo*tide* as opposed to nucleo*side* adducts. Sample stacking in conjunction with CZE–MS for the analysis of DNA adducts was first used for synthetic as well as for in vitro mixtures with CFFAB ionization [11]. Concentration detection limits in the 10^{-8} *M* range with injection volumes as high as 3 μl were demonstrated for target adducts. These numbers represent a nearly 100- to 1000-fold improvement in concentration detection limits.

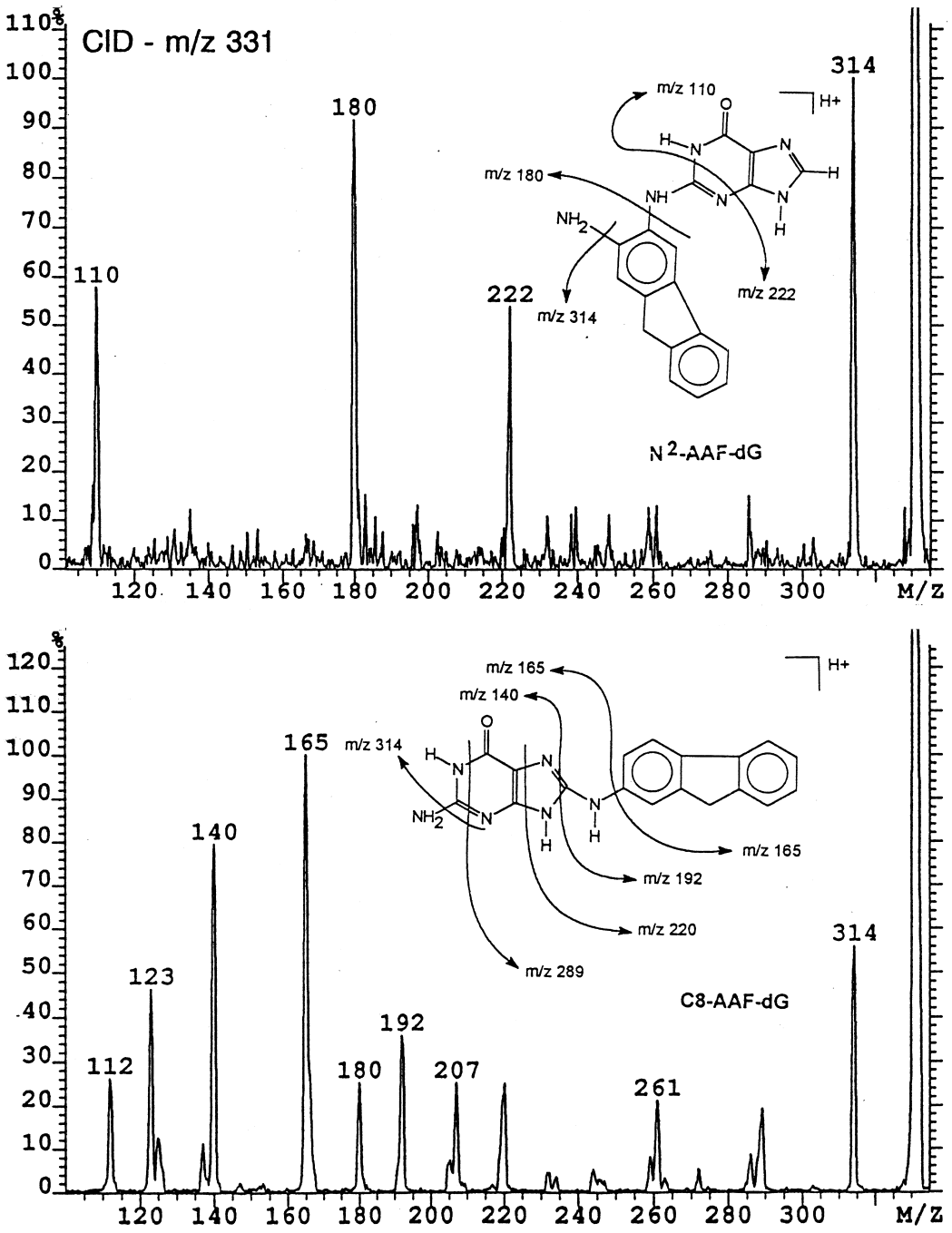


Fig. 8. Collisionally induced dissociation of the *m/z* 331 ion from the FAB spectrum of C8-AAF-dG shown in Fig. 4 (bottom panel) and of the same mass ion formed in the FAB spectrum of its isomeric adduct, N^2-AAF-dG (upper panel). Plausible origin of fragment ions produced by CID shown in the figure. (Susan M. Wolf, Ph.D. Thesis, Northeastern University, 1994.)

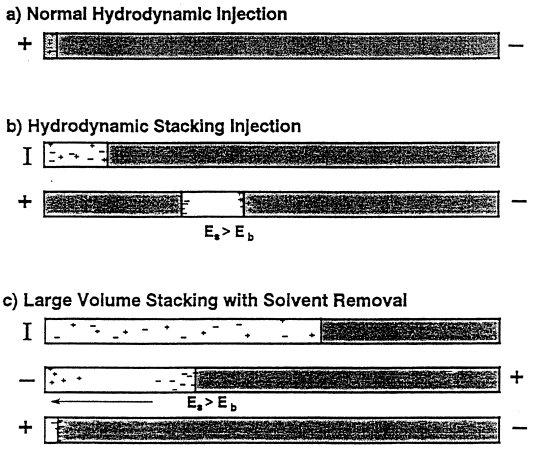


Fig. 9. Sample injection modes in capillary zone electrophoresis. (a) Normal (low volume) hydrodynamic injection; (b) hydrodynamic stacking injection; (c) large volume stacking with solvent removal. Reproduced from Ref. [21] with permission.

Two more recent publications have further explored the utility of sample stacking for DNA adduct detection in CZE–MS using electrospray ionization instead of FAB. In view of the negative charge of the nucleo*tide* adducts, it was natural to use negative ion electrospray (−)ES ionization for detection. Barry et al. [8] and Deforce et al. [22], analyzed deoxynucleotide DNA adducts of benzo[*a*]pyrene and phenyl glycidyl ethers, respectively, from in vitro reaction mixtures. Since much of the work described earlier in this review has dealt with examples involving the fragmentation of positively charged nucleoside adducts, it is of interest to consider the information content of the (−)ES spectra generated from the nucleotide adducts. Fig. 10 [22] compares the CID spectra of the $[M-H]^-$ ions of two isomeric deoxyadenosine monophosphate adducts of phenyl glycidyl ether. Noteworthy is the low relative abundance of the ion produced from the cleavage of the glycosidic bond in the spectrum of the dAMP which is alkylated in the heterocyclic moiety (Fig. 10b). While the low relative abundance of this ion may be attributed in part to the competing formation of the $[B–ArOH]^-$ ion of m/z 190, competition for the charge by the phosphate group to give the S^- ion of m/z 195, probably further diminishes the propensity for charge retention on the purine system. It should be noted that the cleavage of the glycosidic bond was also of reduced prominence in the (−)ES spectra of deoxynucleotide diol epoxide adducts [8]. However, it is unclear whether this is a general trend for the (−)ES spectra of deoxynucleotide adducts since recent observations in our laboratory suggest a more convoluted picture. Clearly, more information is needed before any generalizations —if at all possible— could be made. The mass spectrum of dAMP alkylated on the 5′-phosphate group (Fig. 10a) provides an interesting contrast to its purine-alkylated isomer. The most prominent peak in the CID spectrum is now formed by charge retention with the unsubstituted purine. This pattern further confirms that the purine is not involved in covalent bonding with PGE. Additional fragment ions are formed as indicated on the figure.

Thus far the bulk of the analytical work in the area of DNA adducts has been done by LC–MS rather than CE–MS. The optimum utility of electroseparation methods, however, especially in combination with MS has not as yet been fully explored. Among the reasons often cited for the slow adoption of CE in the analytical laboratory has been the concern with the low injection volume. As noted above, sample stacking offers one simple means of overcoming this problem. However, other methods which utilize preconcentration on top of the CE capillary [23], have been shown to accommodate dramatically higher injection volumes than sample stacking. When these injection techniques become routinely incorporated into the field of DNA adduct analysis, the concern with concentration detection limits should be eliminated. Another recent development in the field of electroseparations is the re-emergence of capillary electrochromatography (CEC). CEC is a hybrid between CE and capillary LC, and offers a number of unique features which have only recently begun to be appreciated. In fact, the applicability of CEC and CEC–MS to the analysis deoxynucleoside adducts of PAHs has been demonstrated in a recent publication [24]. CEC combines many of the features of CE and capillary LC including fast analysis time and low sample consumption. Moreover, its stop/flow capability makes it an ideal candidate for use with ion trap detectors. Its acceptance into the world of hyphenated techniques routinely coupled to MS is only a matter of time at which point its usefulness will be fully assessed.

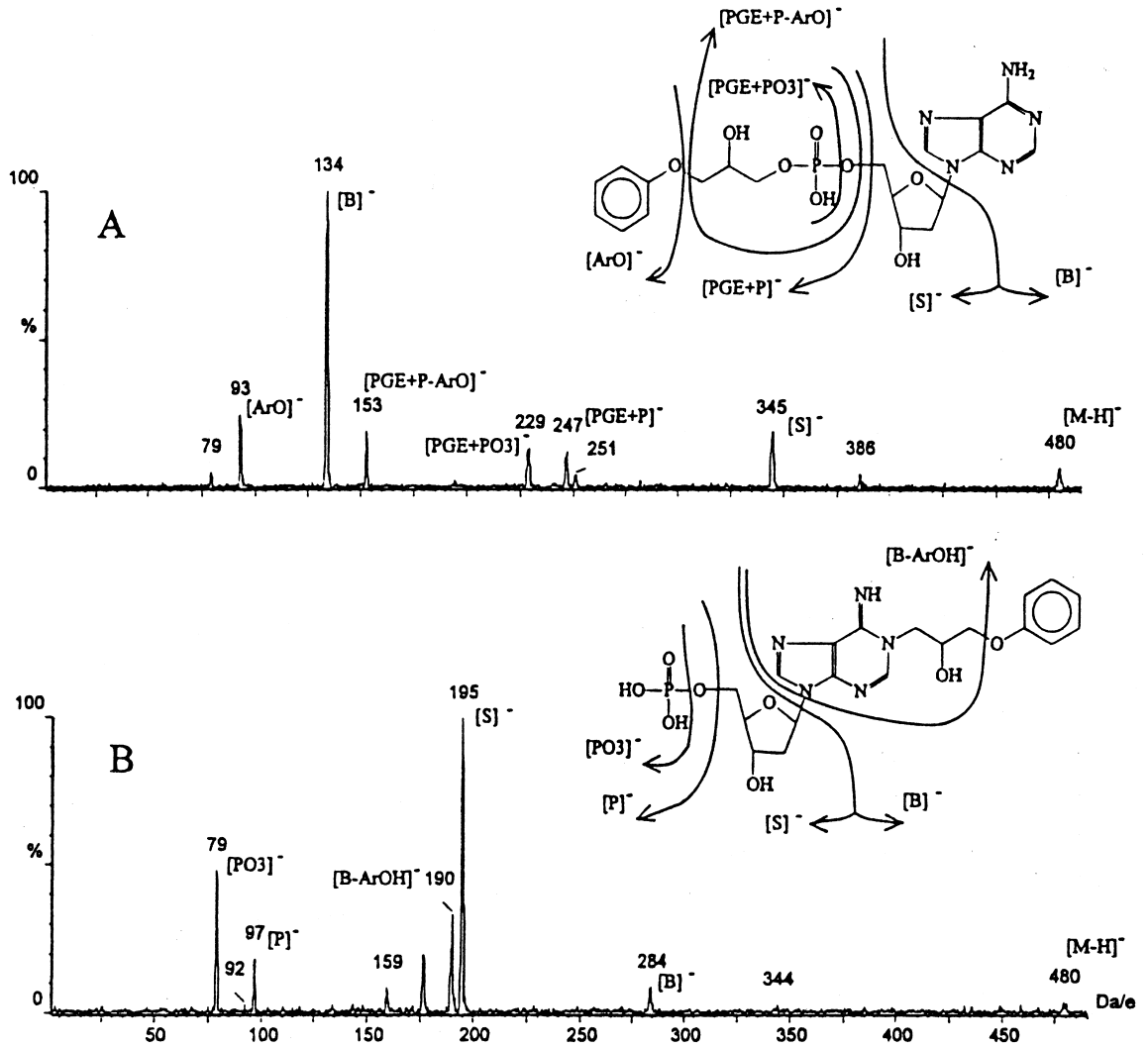


Fig. 10. CID spectra of the parent ions ($[M-H]^-$, m/z 480) of two isomeric PGE adducts of dAMP. (A) Mass spectrum of dAMP with alkylation on the 5′-phosphate moiety. (B) Mass spectrum of dAMP alkylated on the heterocyclic moiety. Reprinted from Ref. [22] with permission.

6. Future prospects

The summary presented above outlines the current status of capillary separation methods in combination with MS in the field of DNA adduct detection and characterization. In reflecting on the levels of detection generally achieved by LC(CE)–MS, it is clear that they are still several orders of magnitude higher than those normally obtained by ^{32}P-postlabeling. As a result, it is unlikely that in the foreseeable future LC(CE)–MS will replace ^{32}P-postlabeling as the routine method of screening for DNA adducts. On the other hand, problems associated with the poor specificity of ^{32}P-postlabeling, the possibility for false positives [25] and its inability to identify unknowns, are pointing to the need for more definitive detection methods. When in vitro reactions are scaled up to levels compatible with LC–MS/MS sensitivity, comparison of data obtained by the two techniques demonstrate the inherent power of the MS

detector to detect and/or identify unknowns not 'brought forth' by the ^{32}P-postlabeling method [6,12]. Recent advances in ES ionization with the development of nanospray techniques that demonstrate detection capability at the sub-femtomole level [26,27] will help to further bridge the gap between MS and ^{32}P-postlabeling. As these techniques become routinely coupled to separation methods that operate in the nanoflow regime (e.g., micro-LC [28], CE [29], or CEC), we are likely to see capillary separation methods coupled to MS used in parallel with P-32 analysis. In the least, MS will still be used to confirm and/or screen the ^{32}P-postlabeling data or vice versa.

7. Notation

MS	mass spectrometry
LC	liquid chromatography
CE	capillary electrophoresis
CZE	capillary zone electrophoresis
CEC	capillary electrochromatography
TS	thermospray
ESI	electrospray ionization
CFFAB	continuous flow fast atom bombardment
CID	collision-induced dissociation
SIM	single ion monitoring
SRM	single reaction monitoring
MRM	multiple reaction monitoring
CNL	constant neutral loss
C8-AAF-dG	N-(deoxyguanosin-8-yl)-N-acetyl-2-aminofluorene (see Fig. 4 for structure)
C8-PhIP-dG	N-(deoxyguanosin-8-yl)-phenyl-imidazo[4,5-b]pyridine (see Fig. 5 for structure)
N^2-AAF-dG	3-(deoxyguanosin-N^2-yl)-2-(acetylamino)fluorene (see Fig. 8 for structure of aglycone part of adduct)
AAAF	N-acetoxy-N-acetyl-2-aminofluorene
MDA	malondialdehyde
dAMP	2′-deoxyadenosine-5′-monophosphate

Acknowledgements

This work was supported by a grant from the National Institutes of Health, 1RO1CA 69390-02.

References

[1] D. Li, M. Wang, K. Dhingra, W.N. Hittleman, Cancer Res. 56 (1996) 287–293.
[2] A. Dipple, Carcinogenesis 16 (1995) 437–441.
[3] K. Randerrath, V. Reddy, R.C. Gupta, Proc. Natl. Acad. Sci. USA 78 (1981) 6126–6129.
[4] E.G. Snyderwine, C.D. Davis, K. Nouso, P.P. Roller, H.A.J. Schut, Carcinogenesis 14 (1993) 1389–1395.
[5] P.B. Farmer, F.M.A. Sweetman, J. Mass Spectrom. 30 (1995) 1369–1379.
[6] R. Singh, G.M.A. Sweetman, P.B. Farmer, D.E.G. Shuker, K.J. Rich, Chem. Res. Toxicol. 10 (1997) 70–77.
[7] J.P. Barry, C. Norwood, P. Vouros, Anal. Chem. 68 (1996) 1432–1438.
[8] R.G. Nath, J.E. Ocando, F.-L. Chang, Cancer Res. 56 (1996) 452–456.
[9] S.M. Musser, S. Pan, P.S. Callery, J. Chromatogr. 474 (1989) 197–207.
[10] S.M. Wolf, P. Vouros, Chem. Res. Toxicol. 7 (1994) 82–88.
[11] S.M. Wolf, P. Vouros, Anal. Chem. 34 (1995) 891–900.
[12] D. Rindgen, R.J. Turesky, P. Vouros, Chem. Res. Toxicol. 8 (1995) 1005–1013.
[13] C.G. Edmonds, M.L. Vestal, J.A. McCloskey, Nucleic Acids Res. 13 (1985) 8197–8206.
[14] T.-Y. Yen, N.I. Christova-Gueoguieva, N. Scheller, S. Holt, J.A. Swenberg, M.J. Charles, J. Mass Spectrom. 13 (1996) 1271–1276.
[15] A.K. Chaudary, M. Nokubo, T.D. Oglesby, L.J. Marnett, I.A. Blair, J. Mass Spectrom. 30 (1995) 1157–1166.
[16] Y. Tondeur, R.C. Moschel, A. Dipple, S.R. Koepke, Anal. Chem. 58 (1986) 1316–1324.
[17] E.G. Rogan, E.L. Cavaliere, S.R. Tibbles, P. Cremonesi, C.D. Warner, L. Nagel, K.B. Tomer, R.L. Cerny, M.L. Gross, J. Am. Chem. Soc. 110 (1988) 4023–4029.
[18] J.A. McCloskey, Chem. Res. 24 (1991) 81–88.
[19] E. Jackim, C. Norwood, J. High Resolut. Chromatogr. 13 (1990) 195–196.
[20] S.M. Wolf, P. Vouros, C. Norwood, E. Jackim, J. Am. Soc. Mass Spectrom. 3 (1992) 757–761.
[21] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046–1050.
[22] D.L.D. Deforce, F.P.K. Ryniers, E.G. Van den Eeckhout, F. Lemiere, E.L. Esmans, Anal. Chem. 68 (1996) 3575–3584.
[23] A.J. Tomlinson, L.M. Benson, S. Jameson, D.H. Johnson, S. Naylor, J. Am. Soc. Mass Spectrom. 8 (1997) 15–24.
[24] J. Ding, P. Vouros, Anal. Chem. 69 (1997) 379–384.
[25] S.V. Vulimiri, C.V. Smith, E. Randerath, K. Randerath, Carcinogenesis 15 (1994) 2061–2064.
[26] M.S. Wilm, M. Mann, Int. J. Mass Spectrom. Ion Processes 136 (1994) 167–180.
[27] R. Korner, M. Wilm, K. Morand, M. Schubert, M. Mann, J. Am. Soc. Mass Spectrom. 7 (1996) 150–156.
[28] R.A. Henderson, A.L. Cox, K. Sakaguchi, E. Apella, J. Schabanowitz, D.F. Hunt, V.H. Engelhard, Proc. Natl. Acad. Sci. USA 90 (1993) 10275–10279.
[29] D. Figeys, I. VanOstveen, A. Ducret, R. Aebersold, Anal. Chem. 68 (1996) 1822–1828.