

A Novel Deoxynucleotide Derivatization Methodology for Improving LC-ESI-MS Detection

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

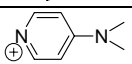
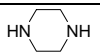



Introduction:

The development of a sensitive LC-UV-MS method for the analysis of DNA nucleotide adducts has been a challenge in bioanalytical chemistry. Detection of DNA nucleotide adducts is complicated by the presence of a native anionic phosphate group. Therefore, nucleotides are analyzed in negative ion mode, which requires basic mobile phases that make chromatographic separation difficult and reduces MS sensitivity. Detection in positive mode would allow use of acidic mobile phases resulting in better chromatography and increased MS sensitivity. We have developed a novel *in-situ* derivatization technique, which attaches a basic group to the phosphate moiety of the nucleotides, creating increased hydrophobicity and increases positive ion mode detection.

Methods:

Coupling reagents typically used in peptide synthesis were used to synthesize several different deoxyguanosine nucleotide phosphoramidates and phosphomonoesters in high yields and under mild conditions. The reactions were carried out at room temperature, overnight in DMSO. The crude mixtures were separated on an Waters Atlantis dC18 50 x 3 mm, 3 μ m column and analyzed by LC-UV-MS via HP1090, fitted with a PDA detector, inline with an LCQ Classic in positive ion mode. The derivatives were characterized by MS/MS and reaction yields determined from the UV traces. Several promising phosphoramidate and phosphomonoester reactions were used to derivatize 8-(N-acetyl-2-aminofluorenyl)-guanosine 5'-monophosphate (C8-AAF-dGMP) adduct which was previously prepared. The C8-AAF-dGMP derivatives were analyzed under the same conditions as the deoxyguanosine 5'-monophosphate (dGMP) derivatives.

Table 1 Validation Parameters for the LC-MS/MS Analysis of dGMP Derivatives

	R	Yield (%)	Monoisotopic MW (da)	Precursor ion [M+H] ⁺	Product ion MS/MS	Retention time (min)
1	OMe	94.1	361.08	362.2	152.1	6.12
2		95.6	397.09	398.2	312.2	10.36 [*]
3		93.7	412.11	412.2	243.3	6.98 [*]
4		61.7	452.14	452.2	283.1	14.14 [*]
5		89.1	415.14	416.2	247.2	4.84 [*]
6		96.3	429.16	430.1	261.0	5.02 ^{***}
7		95.8	414.14	415.2	152.3	7.77 ^{**}
8		95.4	428.16	429.1	152.3	13.1 ^{***}

Solvent A = 10 mM ammonium acetate

Solvent B = methanol

*Method#1: 5/95% B/A (dGp Rt=3.00)

**Method#2: 20/80% B/A

***Method#3: 5%B for 3 minutes, 5-30% B over 5 minutes hold for 7 minutes at 30%.

Total runtime: 20 min

Table 2. Modified dGpAAAF Adducts Area Comparison

Compound	S/N	Rt	MS/MS2	AreaMS	Area MS2	Total	AVG
dGpAAAF	33/7	3.59	569.0/373	27708418	9192176	36900594	
dGpAAAF	37/14	3.57	569.0/373	22729743	10851374	33581117	34500800.7
dGpAAAF	38/13	3.53	569.0/373	21848927	11171764	33020691	
dGpAAAF-CH3	161/35	4.62	582.0/373	39184686	23866743	63051429	
dGpAAAF-CH3	206/28	4.61	582.0/373	42232260	22440997	64673257	63618544.0
dGpAAAF-CH3	168/32	4.62	582.0/373	39280462	23850484	63130946	
dGpAAAF-DMAP	68/3	5.13	673/373	19451689	2105976	21557665	
dGpAAAF-DMAP	68/2	5.1	673/373	19458559	1837127	21295686	21155173.0
dGpAAAF-DMAP	68/2	5.13	673/373	19049326	1562842	20612168	
dGpAAAF-pip	170/33	3.69	636/373	68505703	34150919	102656622	
dGpAAAF-pip	143/29	3.82	636/373	61419981	37430750	98850731	105195706.0
dGpAAAF-pip	164/49	3.74	636/373	72434823	41644942	114079765	

Discussion:

A series of 2-methylimidazolidine, 4-dimethylaminopyridine, imidazolidine, piperidine, piperazine derivatives of dGMP were synthesized. Using these derivatives, for the first time that we are aware of, positive ion mode MS was used to successfully analyze deoxynucleotides. The derivatives were evaluated for ionization efficiencies, fragmentation patterns, and chromatographic reversed-phase properties by LC-ESI-MS/MS. The 4-dimethylaminopyridine and piperidine derivatives of dGMP had synthetic reaction yields in **excess of 95%** and **showed a 4 and 5 fold signal improvement**, respectively, in positive ion mode **versus the underivatized deoxynucleotide** (data not shown). These two derivatives also showed improved chromatographic retention in reverse phase HPLC. When subjected to MS/MS, these derivatives showed specific fragmentation of the nucleobase, which is important for the sensitive detection and identification of DNA adducts in tandem MS.

Based on the results of the dGMP derivatives, we proceeded to evaluate the effect of phosphate derivatives on dGMP adducts. The C8-AAF-dGMP was reacted to form the 4-dimethylaminopyridine and piperidine derivatives and the adducts were also successfully analyzed in positive ion mode. In similar fashion to the dGMP, the derivatized adducts showed improved ionization efficiencies, had diagnostically important fragmentation patterns, and showed increased retention when compared to the underivatized compound. For example, the C8-AAF-dGMP piperidine derivative **showed a 4 fold signal-to-noise enhancement** over the underivatized compound (Table 2). During the course of our investigation we also identified two derivatives that resulted in compounds that possess a fixed positive charge, which we anticipate, will significantly enhance the ionization in positive ion mode while reducing background noise by the elimination of acid in the mobile phase. For the first time we have demonstrated the utility of derivatizing deoxynucleotides for LC-MS analysis in positive ion mode to enhance sensitivity and facilitate chromatographic separation under commonly used reversed-phase chromatography conditions.

Reference:

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- Barry, J. P., Norwood, C., Vouros, P. (1996) Detection and Identification of Benzo[a]pyrene Diol Epoxide Adducts to DNA Utilizing Capillary Electrophoresis-Electrospray Mass Spectrometry. *Anal. Chem.* 68(8), 1432-8.