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Primary contribution of the injector to carryover of a trace analyte in high-performance liquid chromatography

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ABSTRACT

When a low-nanogram amount of N1,N3-bis-(pentafluorobenzyl)-N7-(2-[pentafluorobenzyloxy]ethyl)xanthine was subjected to HPLC, low-picogram amounts of the compound could be detected subsequently (off-line by gas chromatography–electron-capture negative-ion mass spectrometry) after injection of pure mobile phase. This was in spite of significant, intermediate washing of the injector and column. It was determined that essentially 99.9% of this analyte contamination came from the injector. Use of two injectors is a practical remedy for this problem.

INTRODUCTION

It is well known that sample carryover can be a problem in the analysis of trace analytes by HPLC. For example, Lin and Desiderio [1] encountered this difficulty in the separation of neuropeptides by reversed-phase HPLC. Generally the HPLC system is simply washed thoroughly to overcome carryover of analyte, without an effort to determine the mechanism. An exception to this is work which showed that the carryover was due to sample adsorption in the injection loop [2]. In this case, a fixed-volume loop was used in the overfill mode with a weak injection solvent. Similar observations have been made by Simonson and Nelson [3].

We are pursuing the detection of N7-(2-hydroxyethyl)guanine, an ethylene oxide DNA adduct, by gas chromatography–electron-capture negative-ion mass spectrometry (GC–ECNI-MS) [4]. Towards this goal, we are purifying N1,N3-bis-(pentafluorobenzyl)-N7-(2-[pentafluorobenzyloxy]ethyl)-xanthine, a derivative of this analyte, by reversed-phase HPLC prior to its detection by GC–ECNI-MS. As we reported before [4], we encountered analyte carryover of the latter compound in the HPLC system. We circumvented the problem, at least at the 100-pg level of analyte, by purifying the compound instead by solid-phase extraction on silica. However, the overall GC–ECNI-MS chromatograms were cleaner when the samples were first purified by HPLC. This result, plus the fact that we intend to extend the method to lower-analyte levels, has maintained our interest in using HPLC for sample cleanup of this compound prior to its detection by GC–ECNI-MS.

In this paper, we examine the contribution of the injector to this problem of analyte carryover.

EXPERIMENTAL

All the equipment and reagents were the same as reported before [4], except that here we substituted methanol for acetonitrile in the HPLC system since methanol is a lower cost, less toxic solvent.

Experiment I

A 15-ng amount of analyte was injected twice in-

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to the HPLC system with UV detection, to establish the retention time. [Mobile phase, methanol–water, 80:20 (v/v) at 1.0 ml/min; column, Microsorb silica reversed-phase, 150 × 4.6 mm I.D., 10 μm, Rainin, Woburn, MA, USA; injector, Model 7125, Rheodyne, Cotati, CA, USA; retention time, 9.0 min. In all cases, the sample or mobile phase blank was loaded and injected immediately after washing the injector.] A clean injector (previously unused) of the same type was substituted and flow of the mobile phase was continued for 30 min. An aliquot of 50 μl of mobile phase was injected and the appropriate 2-ml fraction was evaporated, redissolved in 10 μl of toluene, and 1 μl was injected into the GC–EC-NI-MS system: observe 16 fg, corresponding to a carryover of 160 fg in the 2 ml fraction (0.000005% of the 30 ng injected originally). After the mobile phase was flowed for 30 min, mobile phase was injected. Carryover: 20 fg/2 ml, which is 8-fold lower than in step 3. A gradient was conducted [methanol–water, 80/20 (v/v) up to 100% methanol in 10 min, hold for 4 min, then return to the original composition in 10 min] and mobile phase was injected. Carryover: none observed (< 1 fg). The prior (ng-exposed) injector was installed and mobile phase was injected. Carryover: 24 pg (0.08% of 30 ng). After the mobile phase was flowed for 30 min, the injector was washed (using a needle port cleaner, part No. 7125-054 from Rheodyne; the injector was flushed in the load position with 3 × 0.5 ml of warm methanol, and similarly in the inject position, and this entire washing procedure was repeated twice), and mobile phase was injected. Carryover: 10.8 pg.

Experiment II

The above step 1 was repeated, using the ng-exposed injector (but which had been cleaned prior to this second experiment by repetition of the above washing procedure until carryover was absent). After the clean injector was re-installed, a mobile phase gradient was conducted as above, and mobile phase was injected. Carryover: 60 fg. The gradient and injection of mobile phase was repeated. Carryover: none detected (< 1 fg). The prior (ng-exposed) injector was re-installed and washed with 2.5 ml of warm methanol in the load position, the same in the inject position, and mobile phase was injected immediately. Carryover: 7.7 pg.

RESULTS AND DISCUSSION

In order to determine the contribution of the HPLC injector to the analyte carryover that we observed, we employed two injectors, one for injection a nanogram amount of analyte to establish its retention time (and for intentional contamination of the HPLC system), and a second, clean injector for performing subsequent injections of pure mobile phase as blanks. Thus any carryover of analyte observed after the second injector was installed would have to arise subsequent to this injector in the HPLC system, presumably in the column.

The two experiments that we conducted, and our results, are presented in detail in Experimental. As indicated, Experiment I establishes that it is the injector which contributes essentially 99.9% of the picogram level carryover arising from the prior nanogram level injections. The mechanism(s) for holdup of this tiny fraction of analyte in the injector were not studied, but must be due to active sites, solvent dead volumes (*e.g.* from crevices and cracks), or both in the injector. In regard to the possible role of active sites, the sample contacts polytetrafluoroethylene, Vespel, alumina ceramic and stainless steel surfaces in this injector according to the manufacturer [5].

In Experiment I, the remaining carryover of analyte, apparently from the HPLC column, disappeared only after a mobile phase gradient was conducted. We wondered whether the gradient *per se* was important, or whether it was just the additional flow of mobile phase with time that cleaned the system. After all, the residual carryover of analyte had already decreased significantly (from 160 to 20 fg) during the prior interval of isocratic elution, and the composition of the mobile phase only underwent a small change (80 to 100% methanol) during the gradient.

We answered this second question in Experiment II, which is also summarized in detail in Experimental. As indicated, conducting a gradient immediately after the installation of the clean injector still gave comparable carryover of analyte (60 fg) relative to that observed in Experiment I. Thus, washing isocratically appeared to be just as effective for cleaning as conducting a gradient.

The total carryover of analyte that we have observed is far below 1% of the originally injected

sample. Most applications of HPLC would not be bothered by this tiny amount of carryover. However, as HPLC increasingly is coupled directly or indirectly to sensitive detectors like GC–ECNI-MS, more workers will need to deal with this event.

The work also suggests a practical remedy for the problem: use two injectors, each dedicated to a different level of analyte. At least for the application presented here, we find this strategy more attractive than the practice of injecting a trace amount of a radiolabeled analyte standard to establish the retention time.

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