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Note

Direct determination of acrylamide in tissue culture solution by liquid chromatography using column switching

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An analytical procedure for the determination of trace levels of acrylamide monomer (propenamide; AAm) in tissue culture solution was required for the development and use of a flow-through *in vitro* skin penetration technique. Applying this technique, a test compound or mixture is placed on a preparation of excised rat skin and penetration of the compound of interest is measured in the tissue culture solution flowing beneath the skin[1].

Although there were examples for the determination of acrylamide in complex biological and/or aqueous matrices[2–4], these methods all require time-consuming sample clean-up, and no specific method existed for this particular determination. A simplified technique was desired due to the expected sample load, however, the many interferences in the tissue culture solution matrix precluded direct quantitation of acrylamide by conventional analytical techniques[5]. By utilizing the different separating characteristics of two distinct liquid chromatographic (LC) columns, column switching[6] provided the selectivity necessary for the direct determination of acrylamide in tissue culture solution at low ppb* concentrations.

This paper describes the instrumentation incorporated in the LC system used for the analysis, and includes validation data for the method.

MATERIALS AND METHODS

Materials

Chemicals used were acrylamide (99+%, electrophoresis grade, Gold Label; Aldrich, Milwaukee, WI, U.S.A.); concentrated sulfuric acid (J. T. Baker, Phillipsburg, NJ, U.S.A.) and glass-distilled, deionized water. The tissue culture solution was minimum essential medium–D-valine (MEM D-VAL; Gibco Labs., Grand Island, NY, U.S.A.). It was buffered at a concentration of 25 m*M* 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES, 99%; Sigma, St. Louis, MO, U.S.A.) and treated with an antimicrobial–antimycotic solution used at 1% strength (500 units penicillin, 500 μg streptomycin and 1.25 μg fungizone per 500 ml tissue

* Throughout the article the American billion (10^9) is meant.

culture solution). The penicillin, streptomycin and fungizone were obtained from Sigma.

Standard preparation

A stock solution was prepared by dissolving a known weight of acrylamide in distilled, deionized water for a concentration of approximately 5000 μg/ml. This stock solution was diluted in water to concentrations in the range of 1.0 μg/ml to 0.001 μg/ml for a series of standards for analysis.

Sample preparation

No sample preparation was necessary; samples were injected directly into the LC system. Some samples were stored frozen and thawed to room temperature prior to analysis.

Preparation of fortified samples

Fortified tissue culture solution samples were prepared by adding an aliquot (usually 20–100 μl) of the appropriate acrylamide standard solution to a 2-ml or 5-ml sample of tissue culture solution. After spiking, the solution was shaken manually to ensure proper mixing.

Instrumentation and chromatography

Fig. 1 contains a schematic diagram of the LC system. A Varian Model 8050 LC Autosampler (Varian Assoc., Palo Alto, CA, U.S.A.) was used to inject samples into the system. Initial sample separation was done on column I, a 10-μm C_{18} Radial-Pak column with a RCSS C_{18} Guard-Pak (in a RCM-100 radial compression module). This column was preceded by a 2.54 × 0.39 cm I.D. guard column packed with Bondapak C_{18}/Corasil 37–50 μm (all from Waters Assoc., Milford, MA, U.S.A.). A six-port valve (Rheodyne Model 7000 ARV switching valve with Model 7001 pneumatic actuator; Rheodyne, Cotati, CA, U.S.A.), in-line between the two analytical columns, was used to transfer automatically a selected fraction (spanning the retention time of acrylamide) from column I onto column II, a 11.0 × 1.3 cm I.D. Aminex 50W-X4 30–35 μm column (Bio-Rad Labs., Richmond, CA, U.S.A.). Normally the valve was switched to "position B" 90 s after injection and then back to "position A" at 230 s (Fig. 1).

The flow of 0.001 *N* sulfuric acid (aqueous; filtered and degassed) mobile phase was maintained through column I at 3.0 ml/min (1000 p.s.i.) and at 1.5 ml/min through column II (< 500 p.s.i.) by a pair of LDC ConstaMetric III pumps (LDC/Milton Roy, Riviera Beach, FL, U.S.A.). When the sample fraction was transferred between columns (valve position B) the flow through column I was temporarily 1.5 ml/min. A pre-column, 2.54 × 0.39 cm I.D. Corasil Type II 37–50 μm (Waters Assoc.), was in-line between the pump delivering 3.0 ml/min and the sample injector.

A Kratos Spectroflow 773 ultraviolet detector (Kratos Analytical Instruments, Ramsey, NJ, U.S.A.) set at 210 nm, 0.005 A.U.F.S. and the time constant equal to 0.45, was used to monitor the effluent from column II. A Spectrum Model 1021A electronic filter (Spectrum Scientific, Newark, DE, U.S.A.) was placed between the detector and recorder to reduce high frequency noise (settings: output gain, 1; cut-off frequency, 0.01 Hz). A Varian A-25 recorder (Varian Assoc.) set at 2 mVfs as well

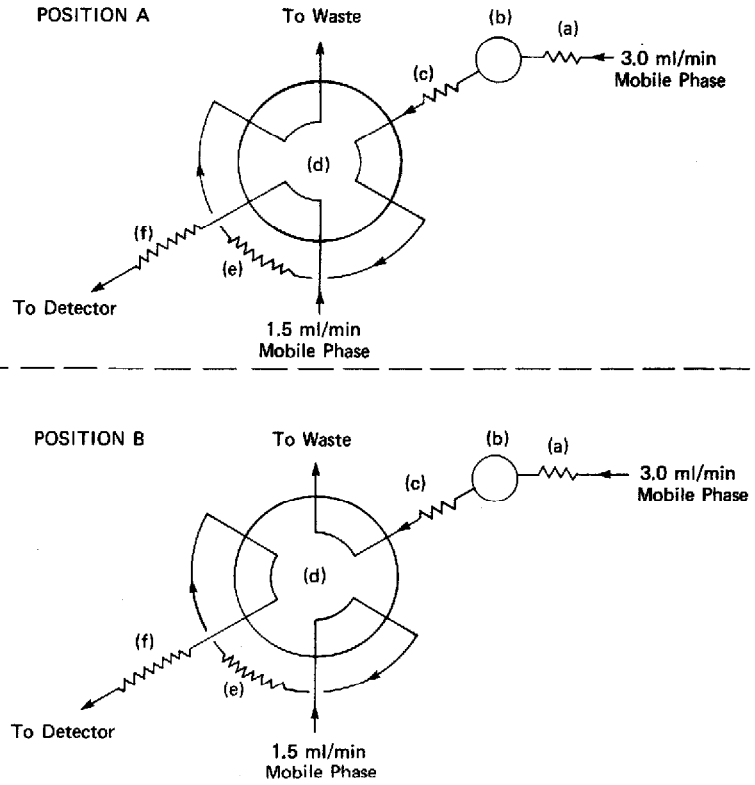

Fig. 1. Schematic of the LC system. Columns and valves: a = pre-column; b = autosampler injection valve; c = guard column; d = column-switching valve; e = column I, 10 μm C_{18} Radial-Pak; f = column II, Aminex 50W-X4.

as a Hewlett-Packard 3354 laboratory automation system (Hewlett-Packard, Palo Alto, CA, U.S.A.) were used for data collection. (The laboratory automation system was also used to trigger the valve switching between analytical columns.) External standards were used to quantitate acrylamide concentrations in samples by peak height measurements.

RESULTS

Chromatography

The chromatographic profiles of blank tissue culture solution samples in contact with rat skin differed from those not in contact with rat skin and varied with time over the course of a 24-h skin penetration experiment. However, no significant interference was observed at the retention time of acrylamide using the column-switching technique.

Sensitivity

The limit of detection for acrylamide standards was 0.005 μg/ml. No response

was recorded for a 0.001-μg/ml standard. The limit of quantitation for acrylamide spikes (in tissue culture solution) was 0.010 μg/ml. The acrylamide peak elutes on the tail of another peak precluding accurate quantitation below that concentration. (See Fig.2 for example chromatograms.)

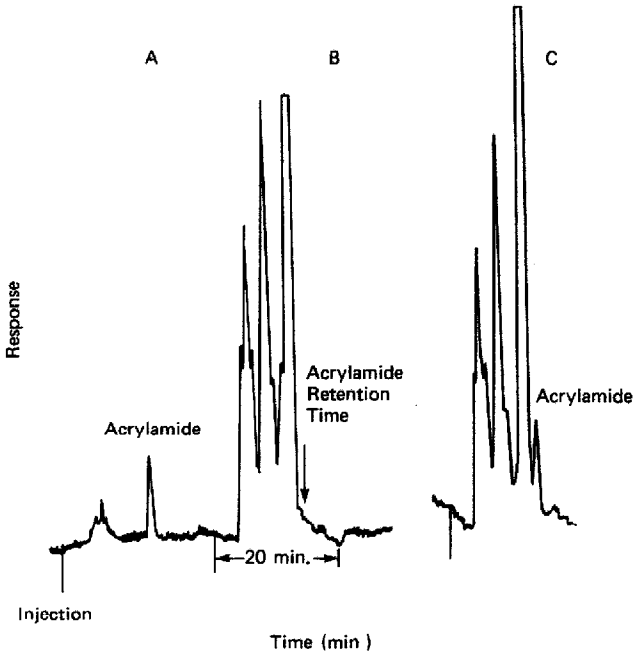

Fig. 2. Example chromatograms: (A) 0.010 μg/ml acrylamide in water standard, (B) Blank pooled tissue culture solution in contact with rat skin, (C) 0.010 μg/ml acrylamide spike in pooled tissue culture solution in contact with rat skin.

Linearity, recovery and reproducibility

The response for acrylamide was found to be linear for both samples and standards over the desired concentration range of 1.0 μg/ml to 0.005 μg/ml for this study. The mean recovery (± standard deviation) of acrylamide from spiked tissue culture solution samples based on five analyses at each of four concentration levels from 1.0 μg/ml to 0.01 μg/ml was 96.7 ± 1.2%. The coefficient of variation ranged from 0.09% at a concentration of 0.998 μg/ml to 2.3% at 0.01 μg/ml. Table I summarizes the AAm recovery data based on peak height measurements.

Stability

Long-term stability of samples was not evaluated for this study. However, fortified samples stored at approximately 1°C for one week or stored frozen for a few days then thawed prior to analysis showed no signs of acrylamide degradation.

TABLE I

ACRYLAMIDE RECOVERY DETERMINATIONS BY PEAK HEIGHT MEASUREMENTS

	Acrylamide concentration (μg/ml)							
	0.010		*0.050*		*0.100*		*0.998*	
	Peak height of standard (cm)*	*Peak height* of spike (cm)*	*Peak height* of standard (cm)*	*Peak height* of spike (cm)*	*Peak height** of standard*	*Peak height** of spike*	*Peak height** of standard*	*Peak height** of spike*
	3.50	3.35	16.44	15.90	5382.91	5017.02	52 362.4	51 180.1
	3.38	3.34	16.30	15.80	5272.30	5142.74	52 857.1	51 264.4
	3.35	3.33	16.30	15.95	5269.57	5076.42	52 482.6	51 219.0
	3.32	3.31	16.52	15.90	5470.45	5106.62	52 824.6	51 177.3
		3.24		15.90		5055.31		51 137.5
Mean	3.29	3.31	16.39	15.89	5348.8	5079.6	52 631.7	51 195.7
S.D.	0.08	0.04	0.11	0.05	96.8	48.0	246.8	48.0
C.V. (%)	2.3	1.3	0.66	0.34	1.8	0.95	0.47	0.09
Recovery (%)***		97.6		96.9		95.0		97.3

* Measured manually.

** Determined using the laboratory automation system.

*** (Mean peak height of spike) − (mean peak height of standard) × 100 = recovery (%).

DISCUSSION

It was found that with constant use, the guard column needed to be changed periodically in order to prevent large, broad peaks from interfering with the analysis. These peaks were assumed to result from the breakthrough of slowly eluting substances that build up on the guard column.

The valve switching times needed to be reestablished each time the guard column was changed, but this could be done in about 1 h. Usually the switching times did not vary more than 30 s with the interval remaining virtually the same.

Peak area measurements were used in early method development. However, peak height measurements were shown to be more accurate for the low level determinations. This was probably due to the fact that the acrylamide peak in fortified tissue culture solution samples elutes on the tail of another peak (unlike standards in water) resulting in less accurate peak integration. A computer program facilitated automated peak height determinations via the laboratory automation system.

CONCLUSIONS

An LC procedure using column switching has been developed and validated for the direct determination of acrylamide in tissue culture solution. This novel analytical approach has provided selective removal of interferences to enable sensitive detection of acrylamide at a relatively non-selective wavelength with no sample preparation necessary. This method will facilitate the evaluation of the skin penetration characteristics of acrylamide monomer and could likely find applications to other complex aqueous matrices.

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REFERENCES

1 J. M. Holland, J. Y. Kao and M. J. Whitaker, *Toxicol. Appl. Pharmacol.*, 72 (1984) 272–280.
2 P. W. Langvardt, C. L. Putzig, J. D. Young and W. H. Braun, *Abstract No. 323 presented at the Eighteenth Annual Meeting of the Society of Toxicology, New Orleans, LA, March 1979.*
3 L. Brown, M. M. Rhead and K. C. C. Bancroft, *Analyst (London)*, 107 (1982) 749–754.
4 C. F. Poole, W.-F. Sye, A. Zlatkis and P. S. Spencer, *J. Chromatogr.*, 217 (1981) 239–245.
5 N. E. Skelly and E. R. Husser, *Anal. Chem.*, 50 (1978) 1959–1962.
6 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, Ch. 16.