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Determination of acrylamide and glycidamide in rat plasma by reversed-phase high performance liquid chromatography

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Abstract

Acrylamide is a widely used monomer that produces peripheral neuropathy. It is metabolized to the epoxide, glycidamide, which is also considered to be neurotoxic. A new reversed-phase high-performance liquid chromatography (HPLC) method is described that permits simultaneous determination of acrylamide and glycidamide in rat plasma. Samples were deproteinized with acetonitrile and chromatography was performed using isocratic elution and UV absorption detection. The limits of detection for acrylamide and glycidamide were 0.05 and 0.25 μg/ml in plasma, respectively, and recovery of both analytes was greater than 90%. The assay was linear from 0.1 to 100 μg/ml for acrylamide and from 0.5 to 100 μg/ml for glycidamide. Variation over the range of the standard curve was less than 15%. The method was used to determine the concentration–time profiles of acrylamide and glycidamide in the plasma of acrylamide-treated rats.

Keywords: Acrylamide; Glycidamide

1. Introduction

Acrylamide (2-propenamide) is a monomer that is widely used in polymer synthesis, water treatment, and electrophoretic separations. Occupational or experimental exposure to acrylamide produces neurotoxicity characterized by ataxia, weight loss, and nerve damage [1]. Acrylamide has also been implicated as a potential mutagen and reproductive toxicant [2–4]. Acrylamide is metabolized to the epoxide, glycidamide (2,3-epoxypropanamide) (Fig. 1), which has been shown to have neurotoxic potential [5]. In light of this finding, it is possible that conversion to glycidamide is an important step in the mechanism

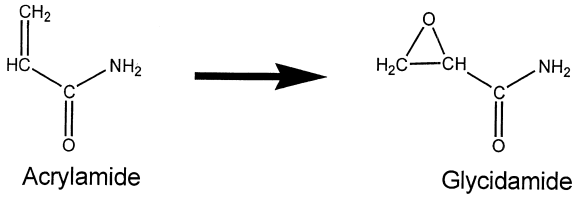

Fig. 1. Structures of acrylamide and its epoxide metabolite, glycidamide.

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of acrylamide neurotoxicity. To investigate this possibility, accurate determination of acrylamide and glycidamide in exposed animals is needed.

The toxicokinetics of acrylamide in rats have been previously determined [6,7], however, determination of glycidamide kinetics has been hampered by the lack of a suitable method for determining its concentration in plasma. Nuclear magnetic resonance (NMR) [8,9], thin layer chromatography (TLC) [6], normal-phase high pressure liquid chromatography (HPLC) [6], and gas chromatography (GC) of derivitized acrylamide [7] have been used to analyze acrylamide in biological samples. Acrylamide and glycidamide adducts on cysteine and valine residues of hemoglobin have also been used as an estimate of exposure to these compounds [10,11]. However, none of these methods have been used for the direct determination of glycidamide in plasma. In this report, we describe a reversed-phase HPLC method that provides simultaneous determination of acrylamide and glycidamide in rat plasma. It is reliable, sensitive, rapid, and utilizes the reversed-phase HPLC methodology found in virtually every analytical laboratory.

2. Experimental

2.1. Chemicals

HPLC grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Acrylamide (>99% pure), acrylonitrile, hydrogen peroxide, and heptane sulfonic acid (sodium salt) were obtained from Sigma (St. Louis, MO). Glycidamide was synthesized from acrylonitrile by reaction with sodium hydroxide and hydrogen peroxide [12]. This method utilized continuous addition of base to reduce decomposition of peroxide and improve selectivity of the reaction for glycidamide compared to a previous method [13]. The identity of glycidamide was confirmed by ^{1}H NMR and ^{13}C NMR spectra (JEOL, 500 MHz) and fast atom bombardment–mass spectrometry (FAB–MS) in a glycerol matrix (VB Fisons, Quattro 4000). Purity of glycidamide was determined to be >95%.

2.2. Chromatography

The HPLC system used consisted of a pump, online degasser, autosampler and diode array detector (1100 series, Agilent Technologies, Palo Alto, CA). A 20-μl aliquot of sample was injected and separation was performed on a 3×250-mm, 5-μm particle size, LUNA C18(2) analytical column (Phenomenex, Torrance, CA), employing a C18 Security Guard™ guard cartridge (Phenomenex). Samples were eluted with a mobile phase consisting of 5 m*M* heptane sulfonic acid in water:methanol (99:1) at a flow-rate of 0.5 ml/min. Detection was performed by monitoring absorbance at 200±8 nm using a reference wavelength of 360±8 nm. Peak area was determined by integration and used to calculate sample concentrations by interpolation from a standard curve extracted from control rat plasma. Standards were prepared by adding 0.1–100 μg/ml of acrylamide and glycidamide to control rat plasma. Recovery was determined by comparing the amount of acrylamide and glycidamide recovered from fortified plasma samples with the quantities of chemical added. Recovery values were averaged across standard concentrations. Inter- and intra-assay coefficients of variation (C.V.) were determined from calculated mean concentrations and standard deviation of each standard from different days and within a day, respectively.

2.3. Animal experiments

Male Sprague–Dawley rats (250–300 g, $n=4$) were treated with acrylamide by a single intraperitoneal (i.p.) injection of 1 ml/kg of a 50-mg/ml solution in distilled water. Blood samples (~200 μl) were collected at specified times by puncturing the lateral tail vein and drawing blood into a syringe containing sodium heparin. Plasma was isolated immediately by centrifuging the whole blood sample at 2000 *g* for 1 min at room temperature.

2.4. Sample preparation

Immediately after isolation of plasma, 300 μl of acetonitrile were added to 100 μl of plasma and mixed for 30 s with a vortex mixer to precipitate the

Table 1
Intra- and inter-assay precision and accuracy data for the determination of acrylamide and glycidamide in plasma

Actual value (μg/ml)	Acrylamide				Glycidamide			
	Observed value (μg/ml)		C.V. (%)		Observed value (μg/ml)		C.V. (%)	
	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter
0.5	0.5±0.1	0.4±0.1	6.1	8.6	0.5±0.1	0.5±0.1	7.5	12.2
1	1.0±0.1	1.0±0.1	3.6	4.8	0.9±0.1	1.0±0.1	4.2	8.3
5	5.1±0.1	5.1±0.3	2.0	5.2	5.0±0.2	5.1±0.2	3.6	4.0
10	10.1±0.3	9.9±0.3	2.6	3.4	10.1±0.2	10.1±0.4	3.2	3.8
25	25.0±0.4	25.1±0.6	1.8	2.5	25.1±0.5	25.0±0.6	2.0	2.6

The accuracy data are expressed as mean±SD (intra-assay, $n=7$; inter-assay, $n=6$).

proteins in the sample. After precipitation, samples were centrifuged for 5 min at 12 000 *g* and the supernatant removed. The resulting pellets were dissociated and washed by adding 300 μl of acetonitrile, mixing for 30 s, centrifuging for 2 min at 12 000 *g*, and collecting the supernatant. Pellets were washed twice in this manner. The pooled supernatants were dried under vacuum at room temperature. Concentrated samples were reconstituted with 100 μl of distilled water. Reconstituted samples were filtered through a 0.22-μm polytetrafluoroethylene (PTFE) syringe filter and analyzed by HPLC.

3. Results

Resolution and sensitivity were determined by injection of standards extracted from control rat plasma. At a flow-rate of 0.5 ml/min, the retention times of glycidamide and acrylamide were ~4.1 and 5.4 min, respectively. Resolution was determined using the formula $R = (t_2 - t_1)/0.5*(w_2 + w_1)$ where R is resolution, t is retention time, and w is peak width. Resolution of acrylamide and glycidamide was calculated to be 3.2. Using a 20-μl injection volume, the assay had a limit of detection of 0.25 μg

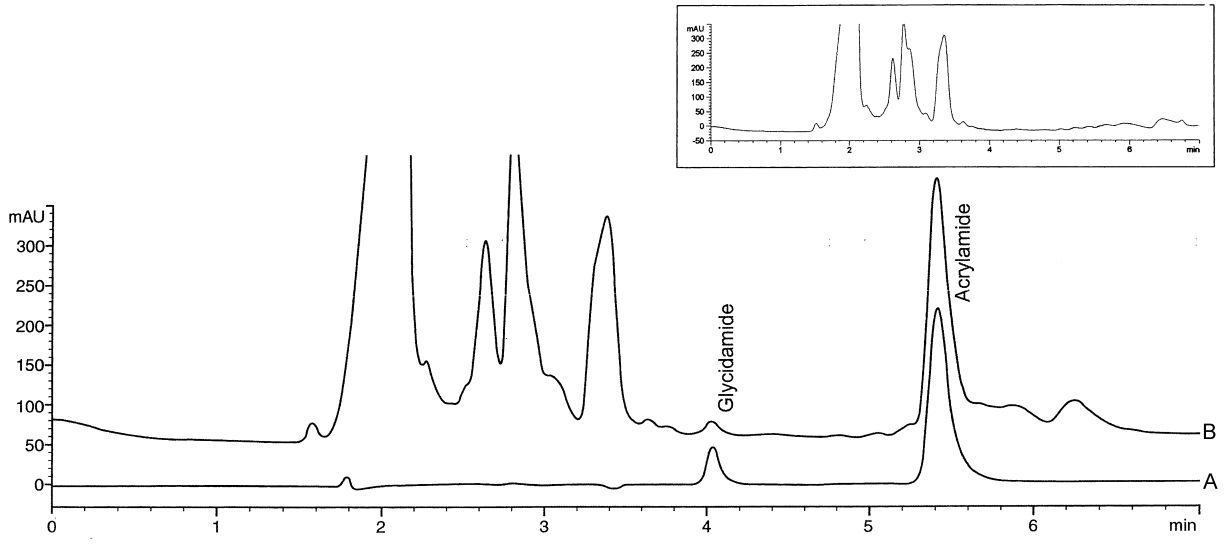

Fig. 2. Chromatogram A is a sample containing 5 μg/ml each of acrylamide and glycidamide in water. Chromatogram B is a plasma extract of a rat 240 min after it received a single 50-mg/kg intraperitoneal injection of acrylamide. The inset chromatogram is control rat plasma.

glycidamide/ml and 0.025 μg acrylamide/ml (at a signal-to-noise ratio of 2.5). The assay was linear between 0.1 and 100 μg/ml for acrylamide and 0.5 and 100 μg/ml for glycidamide. A calibration curve was constructed from peak area versus concentration for each analyte. Correlation coefficients (r^2) for both analytes were greater than 0.99. Recovery for glycidamide was ~92% and that for acrylamide was ~91%. Intra- and inter-assay variation was less than 10% (Table 1). Chromatograms of acrylamide and glycidamide standards (Fig. 2, chromatogram A) and plasma extracts of rats treated with 50 mg acrylamide/kg by intraperitoneal injection (Fig. 2, chromatogram B) are shown. Because of possible interference by plasma components at 200 nm, a representative chromatogram from control rat plasma is also presented (Fig. 2, inset). Concentration–time profiles of acrylamide and glycidamide in the plasma of rats that were treated with 50 mg acrylamide/kg by intraperitoneal injection were determined (Fig. 3). The half-life of acrylamide was determined to be 169 min by linear regression of data from 50–250 min.

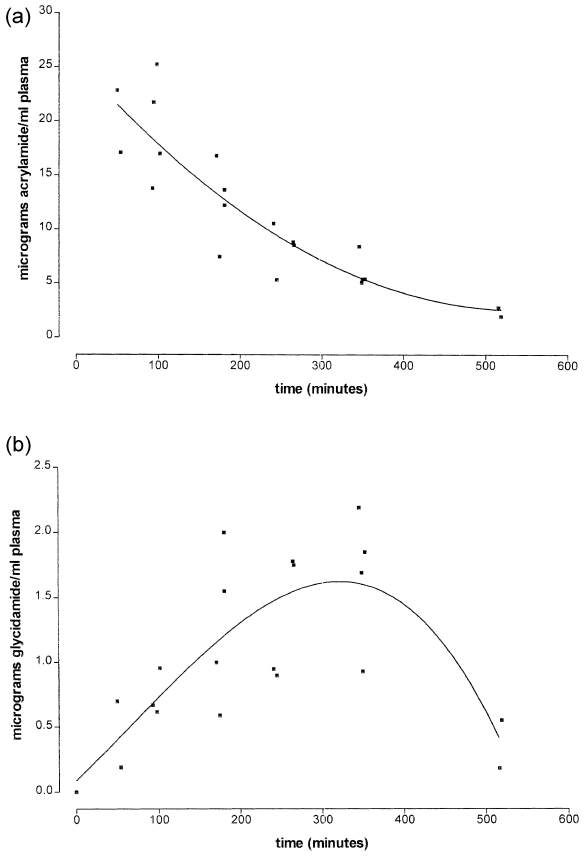

Fig. 3. Concentration-time profile of (a) acrylamide and (b) glycidamide in rat plasma after a single 50-mg/kg intraperitoneal injection of acrylamide. Samples were taken from rats at ~50, 90, 180, 240, 360, and 510 min after injection. Solid line is best fit line of data.

4. Discussion

A reversed-phase HPLC method is described that allows simultaneous determination of acrylamide and glycidamide and provides baseline resolution using 7-min runs. The assay provides excellent recovery and is linear over a wide range of analyte concentrations. Acrylamide and glycidamide are reactive compounds and will bind readily to sulfhydryl groups on proteins. The immediate isolation of plasma followed by precipitation of proteins with acetonitrile used in this method appears to limit loss of analytes to binding with plasma proteins. Sample loss could also occur by volatilization during evaporation. The high recovery of analytes indicates that there is little loss during this step and is consistent with a previous report that found only a 2.5% loss of glycidamide in a solution heated at 100°C for 1 h, suggesting low volatility for this compound [13].

The method was used to obtain concentration–time profiles for acrylamide and glycidamide in the plasma of rats treated with acrylamide (Fig. 3). The half-life of acrylamide in plasma following a single 50-mg/kg injection was determined to be ~2.8 h. This is longer than the 1.7–1.8 h reported by other investigators [6,7]. Previous studies assayed serum and blood, so it is possible that half-life in plasma is longer.

Greater variability was observed in glycidamide than in acrylamide data from treated animals. Inclusion of an internal standard could reduce the variability of the assay, however butyramide and acrylonitrile were tested and found to have unsatisfactory retention characteristics. Plasma concentrations of glycidamide were near the lower limit of linearity for the assay and may have contributed to the variability in this data. The variability observed in the acrylamide data is most likely due to inter-animal differences in acrylamide metabolism. If

necessary, the sensitivity of the assay could be improved by increasing the sample concentration factor or injection volume. If the short wavelength (200 nm) used for detection produces problems with selectivity, use of [^{14}C]acrylamide and radiochemical detection, which are readily adaptable to this methodology, provide a remedy. However, the method as described here allows rapid, simple and accurate determination of acrylamide and glycidamide in plasma using reversed-phase HPLC, making it useful for pharmacokinetic studies.

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