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Note

High-performance liquid chromatographic method for quantitation of acrolein in biological samples

P J BOOR* and G A S ANSARI

Chemical Pathology Laboratory, Department of Pathology, The University of Texas Medical Branch, Galveston, TX 77550 (U S A)

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Acrolein (prop-1-en-3-one) is an extremely reactive α,β-unsaturated aldehyde which has been utilized as a tissue fixative [1]. As a potential industrial respiratory toxin and sensory irritant [2, 3], acrolein has also been shown to have sympathomimetic and cardioinhibitory effects in experimental animals [4] at levels of exposure which have been found in tobacco smoke [5]. Other studies have implicated acrolein as a toxic metabolite in the biotransformation of natural polyamines [6], cyclophosphamide [7, 8] and other anti-tumor drugs [9], allylalcohol [10, 11] and the cardiovascular toxin, allylamine [12].

Acrolein in biological samples has generally been measured by using colorimetric [13, 14] or fluorometric [15] methods. Recently, Patel et al [11] quantitated acrolein by trapping with 2,4-dinitrophenylhydrazine (DNP) followed by high-performance liquid chromatography (HPLC) on a normal-phase column and detecting the DNP—acrolein adduct at 254 nm. The detection limit of their method was 30 ng which can be increased to the picogram level by using an electrochemical detector [16]. In this paper we describe an improved HPLC method with ultraviolet detection of acrolein in the 1-ng range in biological tissues.

EXPERIMENTAL

Chemicals

Acrolein containing 0 05—0.15% hydroquinone as a stabilizer was purchased from Eastman (Rochester, NY, U.S.A.). 2,4-Dinitrophenylhydrazine was

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obtained from Sigma (St Louis, MO, U.S A.). All other solvents and reagents used were of high purity grade.

Synthesis of DNP—acrolein adduct

2,4-DNP was added to 2 *M* sulfuric acid until saturated The yellow solution thus obtained was mixed with acrolein (4 1, v/v) and stored overnight at 4°C The yellow crystals formed were filtered and recrystallized twice from chloroform The structure of the adduct was confirmed by obtaining a 20-eV electron-impact (EI) mass spectrum with a Finnigan 3200 using a solid-probe technique

Recovery experiments

Whole rat liver and kidney were homogenized in 0.25 *M* sucrose (1 10, w/v) Homogenate (0.5 ml) was added to 1.0 ml of phosphate buffer (pH 7 4) and then incubated with 8 mg acrolein and 1.5 ml of 2,4-DNP in acid by three different methods (1) DNP was incubated for 5 min with homogenate, followed by addition of acrolein, (2) DNP was incubated for 5 min with acrolein, followed by addition of homogenate, or (3) homogenate and acrolein were incubated for 5 min, followed by addition of DNP. The DNP—acrolein adduct was then extracted from the homogenate mixture with chloroform, washed twice with 3.0 ml of 2 0 *M* hydrochloric acid and with 5.0 ml of distilled water, dried under nitrogen and redissolved in 1 ml of methanol for HPLC analysis.

Acrolein standards used to analyze recovery were prepared in phosphate buffer (in the absence of homogenate) and were reacted with 2,4-DNP as in the initial synthesis of the 2,4-DNP—acrolein adduct described above Standards were extracted twice in 3 ml of chloroform, dried under nitrogen and redissolved in 0.5 ml methanol for HPLC analysis

High-performance liquid chromatography

HPLC was performed at room temperature (25°C) on a Beckman isocratic liquid chromatograph, Model 330, with an Ultrasphere ODS column (5 μm particle size, 25 cm × 4.6 mm I D) purchased from Beckman Instruments (Berkeley, CA, U S.A.). Acetonitrile—water (1 1, v/v) was used as solvent at a flow-rate of 1 ml/min. The column effluent was monitored at 356 nm A volume of 20 or 35 μl was injected in each case.

RESULTS AND DISCUSSION

The DNP—acrolein adduct gave a retention time of 18—19 min under the isocratic conditions described under Experimental. A linear standard curve was obtained with the curve equation $y = 0.0346x - 0.023$ where y is acrolein (ng) and x is peak height (cm), $r = 0\ 99$ (see Table I).

Recovery of acrolein from homogenates of liver and kidney varied between 5 and 44% as shown in Table II. Fig 1 shows typical chromatograms of the DNP—acrolein standard and acrolein recovered from liver homogenate (approx. 9% recovery). The recovered material from the homogenates appeared to consistently have slightly shorter retention times than acrolein standards

TABLE I

STANDARD CURVE OF ACROLEIN

Data from a typical standard curve are shown, mean peak heights ± S D for three determinations at each acrolein concentration are given (r = 0 99)

Acrolein (ng)	Peak height (cm)
0 58	17 63 ± 1 50
0 45	14 67 ± 0 50
0 35	11 30 ± 0 66
0 23	7 43 ± 0 12
0 12	4 23 ± 0 06

TABLE II

RECOVERY OF ACROLEIN FROM TISSUE HOMOGENATES

Method*	Recovery (mean ± S E M **) (%)	
	Liver homogenate	Kidney homogenate
1	31 2 ± 2 5	26 6 ± 2 6
2	43 8 ± 0 7	38 7 ± 0 7
3	4 6 ± 1 3	9 1 ± 1 5

*Methods 1—3 are specified under *Recovery experiments*
**S E M = Standard error of the mean

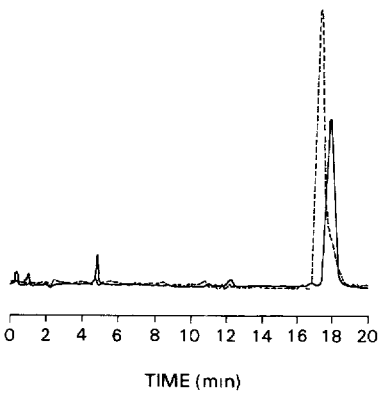

Fig 1 Chromatograms of standard of the acrolein—DNP adduct (solid line) and material isolated from liver homogenates (dotted line), experimental details are described under Experimental A 20-μl aliquot of each sample was injected

Mean (± S D) retention time for the acrolein standard was 18 6 ± 0 14 min (n = 9), whereas acrolein recovered from liver homogenate (n = 14) had a retention time of 18 12 ± 0.62 min and from kidney (n = 12) a retention time of 17 86 ± 0.62 min.

To confirm the identity of the DNP—acrolein adduct extracted from the tissues we collected the effluent from HPLC and compared the mass spectrum of isolated and standard adduct EI (20 eV) mass spectra are shown in Figs

2 and 3. Both spectra gave a base peak at *m/e* 236 corresponding to the molecular ion of the DNP—acrolein adduct. Other significant peaks were at *m/e* 219 [M − OH], 201 [M − (OH + H_2O) or *m/e* 219 − H_2O], 189 [M − CH_2=CH_2—CH_2—OH], 159 [*m/e* 189 − NO], and 142 [*m/e* 159 − OH] [17]. The mass spectrum of the isolated DNP—acrolein adduct shows some contamination (i.e. *m/e* 250, 261, 279 etc) which either may be arising from non-UV-absorbing compounds from tissue preparation having this same retention time or may be residual impurities in the solvent which might have concentrated during solvent evaporation

The recovery from tissue homogenates varies from fair to poor. Acrolein is a very reactive aldehyde and apparently reacts with cell constituents or is metabolized by them very rapidly, as indicated by the lower recoveries obtained by method 3. Method 1, in which acrolein is added after DNP, may result in competition between DNP and tissue homogenate for the highly reactive acrolein and in improved recovery. Method 2, in which DNP and acrolein are combined before addition of tissue homogenate, may provide an opportunity for the DNP—acrolein adduct to form, but apparently the reaction is not complete in 5 min and the remaining acrolein competes for binding between DNP and tissue homogenate.

This method offers advantages over the earlier DNP—acrolein quantitation methods described by Patel et al. [11] By monitoring the adduct at 356 nm, the sensitivity of detection increases by 300-fold but remains less than when using electrochemical detection [16]. The present method utilizes a reversed-phase

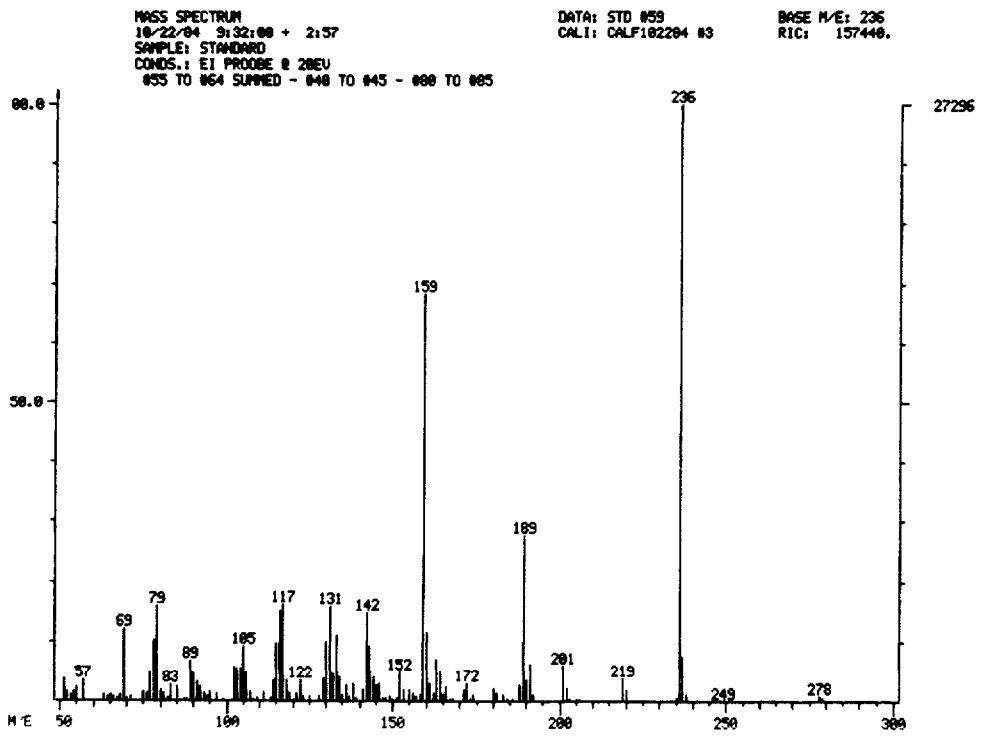

Fig 2 EI (20 eV) mass spectrum of standard of the acrolein—DNP adduct

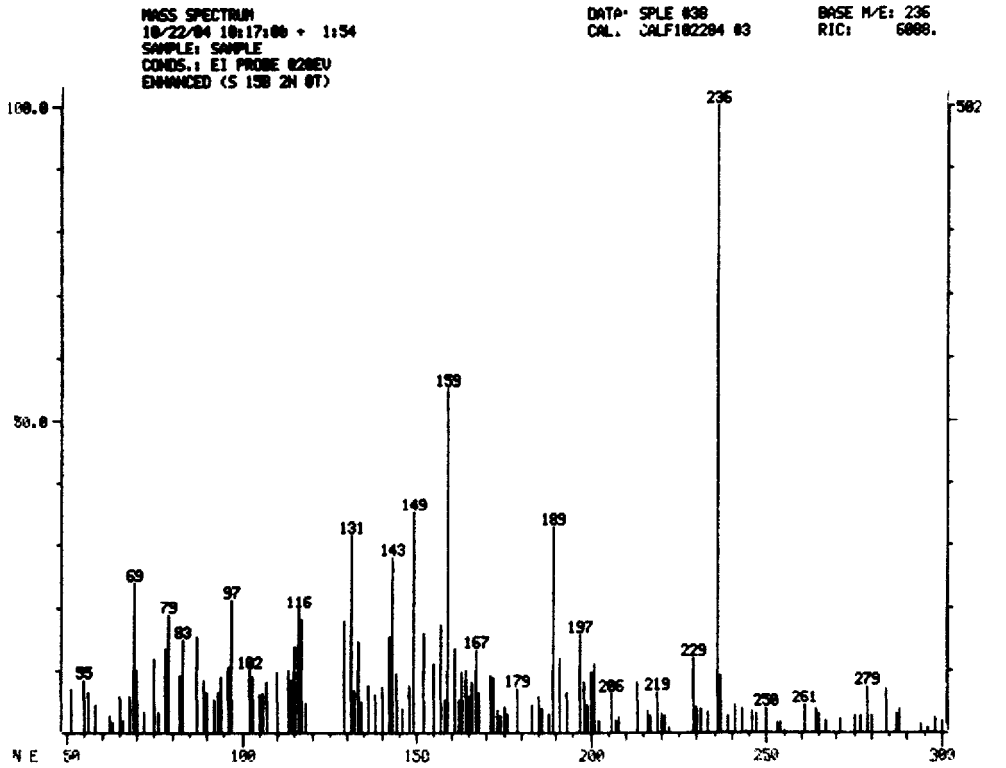

Fig 3 EI (20 eV) mass spectrum of isolated material from liver homogenate

column rather than normal-phase columns as used earlier Since biological material contains many polar compounds, which will elute before the DNP adduct, there will be a gradual accumulation of these polar compounds on a normal-phase column which will result in the loss of efficiency of separation.

In summary, an accurate and sensitive HPLC method for detecting and quantitating acrolein, based on the reaction with 2,4-DNP, has been developed The method can be used for individual biological applications.

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