Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection

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Abstract

An HPLC method was developed for the determination of formaldehyde in human blood plasma. The method was based on the determination of the fluorescent product of the chemical reaction between formaldehyde and ampicillin. A 0.2-ml aliquot of blood plasma was reacted directly with ampicillin under acidic and heating conditions. The reaction product was extracted from the matrix with diethyl ether and analyzed by reversed-phase HPLC with fluorescence detection. Recoveries of spiked formaldehyde at the low ppm (μg/ml) level were between 93% and 102% with relative standard deviations less than 8%. The limits of detection and quantitation of formaldehyde in blood plasma samples were 0.46 μg/ml and 0.87 μg/ml, respectively.   © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Formaldehyde is present in biological fluids or tissues and environment as a result of natural processes or from man-made sources. Formaldehyde is present in many interior construction materials of houses, and can be emitted slowly into the air. Formaldehyde is also used in hospitals, research and teaching laboratories as a sterilizing and preserving agent. Formaldehyde is a highly reactive agent which can react with macromolecules in biological systems.

Spectrophotometric methods were used widely for the determination of formaldehyde in air [1,2]. Chromatographic methods were also used for the determination of low levels of formaldehyde and other aldehydes in air, water and tissues [3–7]. Gas chromatography–mass spectrometry (GC–MS) had been used for the determination of formaldehyde in biological tissues [8]. Liquid chromatography (LC) methods for the determination of formaldehyde in blood plasma were rarely reported. In this article, we describe an LC method for the determination of trace level formaldehyde in blood plasma based on a chemical reaction and its fluorescent product of formaldehyde with ampicillin.

2. Experimental

2.1. Materials

Formaldehyde (USP grade, 37%, w/v, in water) solution, ampicillin [D-(−)-α-aminobenzylpenicillin

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anhydrous] were purchased from Sigma (St. Louis, MO, USA). Trichloroacetic acid (TCA) and other reagents were analytical grade. All solvents were HPLC grade (Tedia, Fairfield, OH, USA) and water was Milli-Q deionized water. Human blood plasma of healthy people obtained from the local blood station was stored at $-70°C$.

2.2. Sample derivatization reaction and extraction

An aliquot of 0.2 ml human blood plasma was pipetted into a 2-ml glass vial, to which 0.8 ml water, 0.1 ml ampicillin solution (2.5 mg/ml, in water) and 0.25 ml TCA (20%, w/v, in water) were added. The vial was capped air tightly and vortex-mixed vigorously for 30 s. The vial was heated in a 90°C water bath for 1 h. After cooling to room temperature, the content of the vial was transferred to a 10-ml glass centrifuge tube. The vial was rinsed with 1 ml diethyl ether twice and also transferred to the centrifuge tube. A small amount of sodium chloride (about 0.5 g) was added to the centrifuge tube which was then vortex-mixed vigorously for 1 min. After centrifugation (1000 *g*) for 5 min, the upper layer (organic phase) was transferred to a 5-ml test tube. The aqueous phase in the centrifuge tube was extracted with another 2 ml diethyl ether and the ether extracts were combined. The diethyl ether was evaporated to dryness with a gentle stream of nitrogen. The residue was redissolved in 0.5 ml acetonitrile–water (50:50), ready for LC analysis. To prepare a reagent blank, 0.2 ml water was used instead of the 0.2 ml blood plasma, and the same procedure was followed.

2.3. Liquid chromatographic analysis

The high-performance liquid chromatography (HPLC) system was an HP 1100 HPLC instrument [Hewlett-Packard (HP), USA], which consisted of a pump, an autosampler, a column chamber, a fluorescence detector, and an HP ChemStation for LC system. The column chamber temperature was set at 40°C and the sample volume was set at 10 µl. The excitation and emission wavelengths of the fluorescence detector were set at 346 nm and 422 nm, respectively. The mobile phase was acetonitrile–water (25:75, v/v) with a flow-rate of 1 ml/min. The HPLC column was an HP Zorbax StableBondSB-

C_{18}, 5 µm, 250 mm$\times$4.6 mm (Germany). The peak area was used for quantitative calculation.

2.4. Calibration

Formaldehyde (USP grade, 37%, w/v, in water) was daily diluted with water to prepare working standards of different concentrations. Blood plasma of healthy people obtained from the local blood station was analyzed to determine its free-formaldehyde concentration by the standard addition method. This blood plasma was then used as sample matrix to prepare a calibration curve and as sample matrix to prepare the daily calibration standards.

To prepare a calibration curve, six 0.2-ml aliquots of the blood plasma (with known formaldehyde content) of healthy people were pipetted into six 2-ml glass vials, respectively. Six 0.8-ml aliquots of different concentrations of formaldehyde standard solutions were added, respectively, to the six vials to prepare a series of calibration standards. In addition, 1 ml of water was pipetted into a separate vial as the reagent blank. Then aliquots of 0.1 ml ampicillin solution (2.5 mg/ml, in water) and 0.25 ml TCA solution (20%, w/v, in water) were added to each of the seven vials. The same procedure described in Section 2.2 was then followed. A calibration curve which covered a concentration range of 1.5~15 µg/ml of formaldehyde in blood plasma was therefore prepared. For routine analysis, a one-point (5 µg/ml) calibration in duplicate was prepared daily and used for quantitative calculation of the blood plasma samples.

3. Results and discussion

3.1. The derivatization reaction

LC systems are more popular than GC and GC–MS systems in most biomedical research laboratories. A highly selective LC method for the determination of formaldehyde in biological fluids without interference from other aldehydes like acetaldehyde would be desirable. In our previous research in establishing an LC method for trace residues of ampicillin in animal tissues, we used a reaction between ampicillin and formaldehyde to form a fluorescent derivative which was quantitatively pro-

portional to the amount of the ampicillin [9]. In the reaction, formaldehyde quantitatively contributed a methyl group to the chemical structure of the fluorescent derivative product [9,10]. Therefore, it was possible to use the same reaction to form a fluorescent derivative which could be quantitatively proportional to the trace amount of formaldehyde in biological fluids.

In our preliminary study, a series of formaldehyde solutions (at the μg/ml level) of different concentrations were reacted with an excess amount of ampicillin, and the products of the reaction were analyzed by HPLC with fluorescence detection. One of the chromatographic peaks was found proportional to the amount of formaldehyde. The UV spectrum of the chromatographic peak was recorded at the same time by a photo diode array detector. The fluorescent product has the same UV absorption spectrum, fluorescence excitation and emission spectra, and LC retention time when compared to the fluorescent product previously reported [9,10].

The chemical reaction was then applied to blood plasma for the determination of trace level formaldehyde. An aliquot of 0.2 ml blood plasma was reacted directly with excess amount of ampicillin under the conditions of heat (90°C) and acid (TCA). The fluorescent derivative was extracted with diethyl ether and then analyzed by C_{18} reversed-phase LC with fluorescence detection. The selection of the reaction conditions such as the amount of ampicillin and TCA was based on the preliminary study in which different levels of ampicillin and TCA were tried and optimized.

3.2. The calibration and the limit of detection

The concentration of formaldehyde in the blood plasma of healthy people which was used as standard matrix for preparing the standard curve was 1.65 μg/ml determined by the method of standard addition. The concentration of formaldehyde in the stored (−70°C) blood plasma of healthy people was recalibrated by the same method every week.

Both calibration lines using water and blood plasma as matrix were prepared. The results indicated that both calibration lines were linear but there was a significant difference between the slopes of two regression equations. Therefore, the calibration line using plasma of healthy people as matrix was

selected and used as calibration line for the determination of formaldehyde in human blood plasma.

The actual concentrations of formaldehyde of the six calibration standards were 1.65, 3.90, 6.15, 8.85, 10.6 and 16.0 μg/ml, respectively. The regression equation of the calibration curve was $y = a_1 x + a_0$ with a linear regression coefficient (γ) of 0.997, where y is the peak area count (mV) of the fluorescent derivative of formaldehyde and x is the concentration (μg/ml) of formaldehyde in blood plasma sample. The coefficients a_1 and a_0 were 103 and 11.8, respectively. The confidence interval ($\alpha = 0.05$) of the intercept (a_0) of the regression equation was calculated to be 11.8 ± 50.2, which includes the point of 0. Therefore, in routine analysis, a one-point calibration in duplicate could be used instead of a calibration curve. But a new calibration curve should be prepared to check the instrumental system in case of any deviation.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the confidence intervals of the regression line of the calibration line (depicted in Fig. 1) following the method described by Meier and Zünd [11]. The LOD and LOQ of the method were 0.46 μg/ml and 0.87μg/ml, respectively.
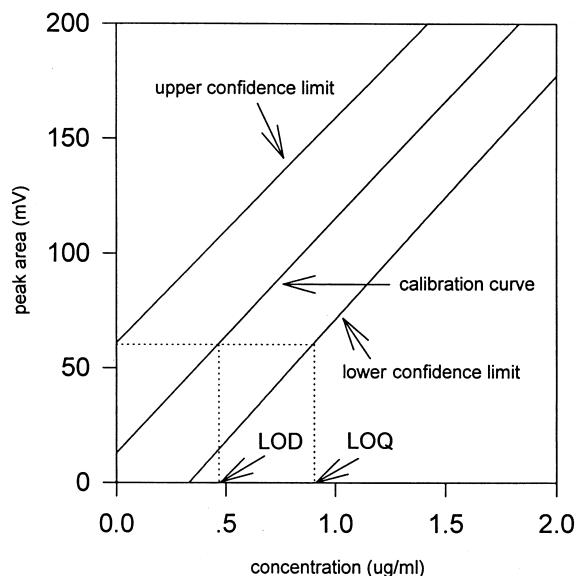

Fig. 1. Determination of the limit of detection (LOD) and limit of quantitation (LOQ) from the calibration curve regression line and its confidence limits.

3.3. HPLC analysis

The chromatographic peak of the formaldehyde fluorescent derivative was well separated from other peaks of the sample (Fig. 2). The formaldehyde derivative was eluted from the HPLC column at the retention time of 8 min. Acetaldehyde and other aldehyde compounds did not interfere with the analysis of formaldehyde. In fact, no specific chromatographic peak was detected when acetaldehyde was reacted with ampicillin with the same reaction conditions. No buffer and pH control were required for the LC mobile phase.

3.4. Method recoveries and variations

Blood plasma samples were spiked with a known amount of formaldehyde at two levels of 1.13 and 2.25 µg/ml. Five replicates of the unspiked blood plasma and spiked blood plasma samples were analyzed in 1 day to evaluate the within-day recoveries and variations. Spiked and unspiked blood plasma samples were also analyzed on different days

to evaluate the day-to-day variations. The data of recoveries and variations are summarized in Tables 1 and 2. The background values of the plasma in Tables 1 and 2 were different to each other and also different to that for the calibration curve, because different batches of plasma were used for the preparation of the calibration standard curve and for the determinations of spiked recoveries.

The average recoveries were between 93 and 102% with relative standard deviations (RSDs) less than 8%. These data indicated that the method was of good accuracy and good precision in the determination of formaldehyde in plasma samples.

4. Conclusion

A highly selective reversed-phase HPLC method for the determination of formaldehyde in plasma was developed. The sample preparation method and derivatization method were relatively simple and straightforward, and no extensive clean-up procedure was needed. The method described was precise and accurate. The method was successfully used to determine formaldehyde in blood plasma samples.

Table 1
Recoveries of formaldehyde from the spiked blood plasma samples (within-day)

Spike level (µg/ml)	Concentration (µg/ml)		Recovery (%)	n	RSD (%)
	Calculated	Determined			
0	–	1.34	–	5	4.4
1.13	2.47	2.33	94.1	5	3.6
2.25	3.59	3.35	93.3	5	6.2

Table 2
Recoveries of formaldehyde from the spiked blood plasma samples (day-to-day)

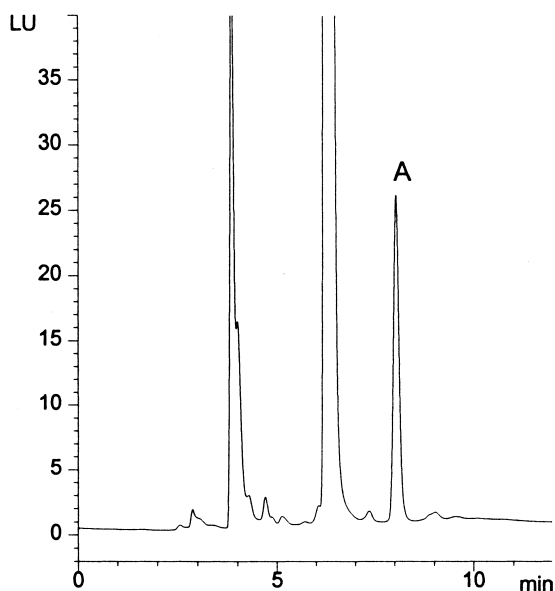| Spike level (µg/ml)	Concentration (µg/ml)		Recovery (%)	n	RSD (%)
	Calculated	Determined			
0	–	0.87	–	3	7.7
1.13	2.00	2.04	102	3	3.1
2.25	3.12	3.07	98.5	3	4.0

Fig. 2. The chromatogram of a typical blood plasma sample indicated the peak (A) of the formaldehyde fluorescent derivative was well separated from other peaks.

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