A Sensitive Micro Method for the Analysis of Benzene in Blood

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A recent communication (ANGERER et al., 1973) has reviewed the requirement for the sensitive and reliable estimation of volatile organic vapors in blood. Threshold Limit Values for the chronic exposure of industrial workers have been established for a variety of hazardous volatile organic substances (AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS, 1971). Many of these may be detected in the circulating blood and the results provide evidence of exposure and, in some cases, the extent of intoxication. Thus, there is a continuing need for sensitive micromethods for the quantitative analysis of low concentrations of volatile organic compounds in blood and other biological fluids. Such methods can then be utilized for the monitoring of exposed human populations by the acceptable and convenient finger puncture sampling technique. Micromethods are also of value in determining blood level curves from small animals which have a limited total blood volume.

The principle of the "head space" analytical technique (CURRY et al., 1962) for the quantitative analysis of volatiles in biological fluids relies on the precipitation of cellular and protein material and the generation of an equilibrated atmosphere within a gas-tight vessel. The subsequent determination of the concentration of molecular species in the equilibrated vapor and its relation to the original concentration in the liquid sample is then usually accomplished by the withdrawal of a precise volume of the vapor with a gas-tight syringe and the injection of the sample into a gas-liquid chromatograph. Peak heights, or some linear derivative, can then be used as a quantitative measure of concentration although deviations from linearity of a plot of peak height against concentration are frequently observed at low concentrations when the peaks are asymmetric. In this communication we report a procedure in which an internal standard of an aqueous solution containing an organic volatile compound of similar physicochemical properties is added to the blood sample. The ratio of peak areas thus generated, as recorded

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by an electronic integrator, then provided a parameter which related linearly to blood concentrations without the requirement for stringent accuracy in the sampling of the vapor volume.

Materials and Methods

0.1 ml of heparinized whole blood, plasma or aqueous sample containing various concentrations of dissolved benzene was placed in a 15 ml septum vial fitted with a screw-cap Mininert™ valve (Precision Sampling Corp., P.O. Box 15119, Baton Rouge, La., 70815, U.S.A.) containing 1.0 ml of a 0.06 M solution of zinc sulfate. 0.22 ml of a 0.28 M solution of barium hydroxide was then added followed by 1 ml of an aqueous internal standard solution of toluene containing 0.017 µg/ml (prepared by dilution of a solution containing 10 µl of toluene per 100 ml water). The Mininert™ valve was found to be especially suitable for this analysis since the small silicone rubber septum was not exposed to the sample vapor until the gas-tight syringe needle was inserted for removal of the vapor sample. The vial and its contents were then incubated at 37°C for 90 min in a Precision Scientific Co. Shaker Bath which reciprocated at 120 oscillations per min. 500 µl of head space vapor were then injected, by means of a gas-tight syringe (Hamilton type 1750-LTN) into a Texas Instruments MT-220 Gas Chromatograph which was connected to a Hewlett-Packard 3370B Electronic Peak Area Integrator.

The chromatographic and integrator conditions were as follows:

Detector: Flame Ionization.

Column: 6', 0.25" I.D., PORASIL TYPE F, 100/120 mesh with Carbowax 400.

Temperatures: Inlet, 90°C; Oven (column), 95°C; Detector, 190°C.

Gas Flow: Carrier Gas (N_2), 38.5 ml/min; Air, 100 ml/min; Hydrogen, 60 ml/min.

Electrometer: Input Attenuation x1; Output Attenuation: x1.

Integrator: Noise Suppression: 3, Slope Sensitivity: UP, 0.3 mV/min, DOWN, 0.03 mV/min, Area Threshold: 10 mV/min.

Results

Solutions for calibration curves were prepared by dissolving 10 µl of pure benzene in 100 ml of plasma or distilled water; 1 ml of this solution was then diluted to 100 ml and solutions containing from 0.0219 to 0.8787 µg/ml were prepared by further dilution. 0.1 ml aliquots of these solutions were treated in the manner described in the methods section and the ratios of the peak area for benzene to that

obtained for toluene were plotted against the known benzene concentration. The results obtained are shown in Table I and the data for human plasma are illustrated in Figure I. The equation of the regression line in Figure 1 was found to be y = 0.2284 x -0.1680 where y is the known concentration of benzene and x is the peak area ratio.

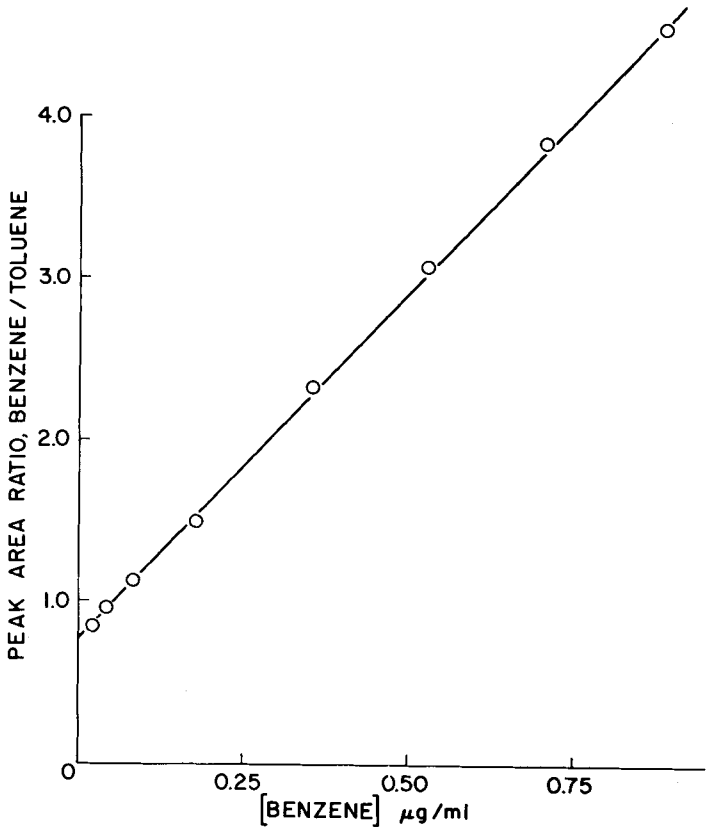

FIGURE 1. PEAK AREA RATIOS DERIVED FROM 0.1 ML SAMPLES OF HUMAN PLASMA CONTAINING BENZENE.

The data in Table I, in agreement with the findings of ANGERER et al. (1973), show that the recovery of benzene from plasma samples corresponded with values obtained from aqueous solution indicating 100% recovery. When aqueous solutions of benzene and the internal standard of toluene, which were made up in 100 volumetric flasks fitted with ground glass stoppers, were stored in the laboratory overnight values of peak area ratios which differed by only 2% from those obtained shortly after preparation were obtained. However, the magnitude of individual peaks for benzene and toluene showed a reduction of about 30% indicating a loss of volatile organic material

TABLE 1.

Peak Area Ratios Obtained from Prepared Solutions of Benzene in Water, Pig and Human Plasma.

[Benzene] µg/ml	Peak Area Ratios, Benzene/Toluene		
	Water	Pig Plasma	Human Plasma
0.8787	4.545	4.550	4.560
0.7030	3.772	3.680	3.820
0.5272	3.102	2.980	3.060
0.3515	2.316	2.308	2.321
0.1757	1.447	1.550	1.493
0.0878	1.152	1.147	1.120
0.0439	0.980	0.951	0.951
0.0219	0.838	0.860	0.829

from solution. Since this clearly could introduce problems, in that an aqueous internal standard would decrease in concentration during the period of a working day, internal standard solutions were made up fresh daily and the solution stored in 5 ml flasks with ground glass stoppers with only a small vapor phase. Each sample containing an unknown concentration was then assayed with the aid of a separate solution of internal standard.

The reproducibility of the method was tested by performing analyses in duplicate for six solutions ranging in concentration from 0.87 to 0.02 µg/ml. The maximum difference between peak area ratios obtained from duplicates was 2.1% with a mean variation of 0.9%. At the lowest concentration assayed this represents a precision of 4×10^{-4} µg/ml for a sample volume of 0.1 ml.

Since the sensitivity and stability of different types of gas chromatographic instruments may vary it is perhaps instructive to report the magnitude of the signal generated by the amount of benzene present in the 500 µl of vapor sample. In concurrent experimentation we obtained calibration curves for standard concentrations of benzene vapor within a chamber. The concentration of benzene vapor in the head space of the septum vial, for the solution known to contain 0.0219 µl of benzene per ml, was found to be 0.1034 ppm. 500 µl of this head space vapor thus contain 1.65×10^{-10} g of benzene (0.165 nanograms) and this yielded an area count of 1740 mV min.

Discussion

Probably the greatest sources of error in a head space analytical technique occur in the sampling of the head space volume with a gas-tight syringe and from the escape or adsorption of vapor from the septum vials during the incubation period. Sampling errors of between 5 and 10% are to be expected if only one detectable component is present in the vapor phase. When an internal standard is added, the peak area ratio remains the same no matter by how much the sample volume is varied and, thus, this source of error is virtually eliminated.

In the method described here, toluene fulfills the requirements of an ideal internal standard for the determination of benzene, and

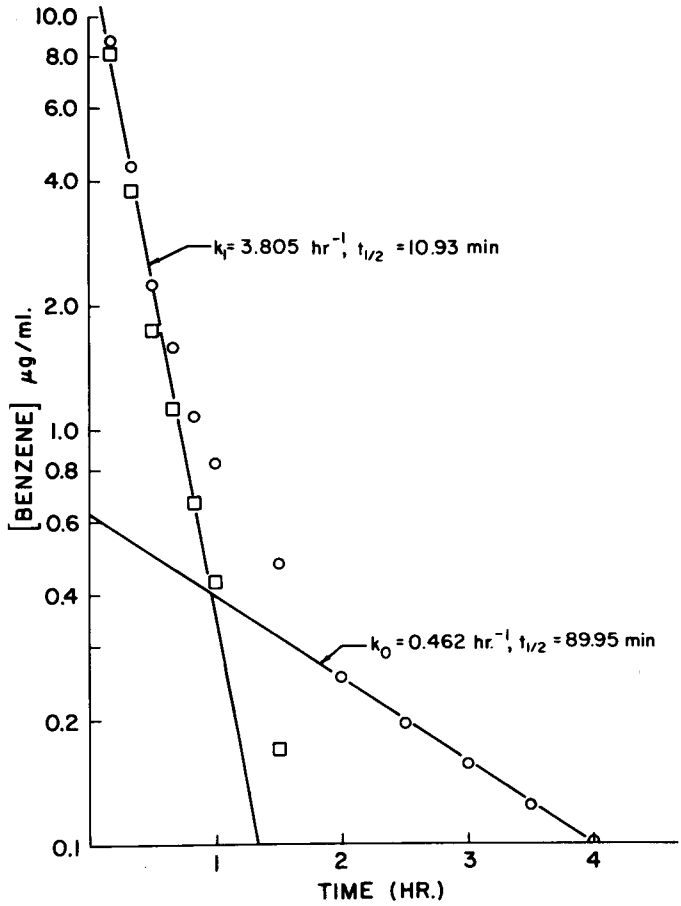

FIGURE 2. BLOOD LEVELS OF BENZENE OBTAINED FROM A 491 g RAT DOSED AT 11.25 µg/Kg (I.V.) ○ MEASURED BLOOD LEVELS. ◘ RESIDUALS OBTAINED BY SUBTRACTING THE VALUES INTERPOLATED FROM THE TERMINAL LINE FROM INITIAL DATA POINTS.

vice versa, in that they are of similar chemical structure and similar physico-chemical properties so that, although their peaks are well resolved they have similar retention times.

An example of the application of this technique is afforded by the data illustrated in Figure 2.

In this experiment a male rat of weight 491 g was surgically prepared by inserting an indwelling cannula into the jugular vein (VAN PETTEN, 1970). 6.3 µl (11.25 µg/kg) of pure benzene was injected (I.V.) via the cannula, and 0.1 ml blood samples were taken every 10 min for the first hour and then every 30 min for the next 4 hours. The one-compartment pharmacokinetic model which fits this data for the elimination of benzene from the blood is clearly bi-exponential of the form:

$$C_t = A_o e^{-k_o t} + A_1 e^{-k_1 t}$$

Where C_t is the blood concentration at any time, t, k_o and k_1 are the rate constants for the initial and final elimination rates and A_o and A_1 are the pre-exponential constants.

The excellent linearity of the terminal points on the semi-logarithmic plot is derived from blood levels of benzene which are less than 0.2 µg/ml. The feathering of the initial points, by subtracting values interpolated from the terminal line from the actual data points, allows the determination of both kinetic coefficients for the elimination process.

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