Journal of Chromatography, 188 (1980) 412–416

CHROM. 12,409

Note

Determination of halogenated anilines in urine by high-performance liquid chromatography with an electrochemical detector

E. M. LORES, F. C. MEEKINS and R. F. MOSEMAN

Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711 (U.S.A.)

(Received September 17th, 1979)

This paper describes a method for the determination of halogenated anilines in urine using high-performance liquid chromatography (HPLC) and an electrochemical detector. The electrochemical detector provides the sensitivity needed to determine anilines at subnanogram levels and HPLC provides a method of chromatography that does not require derivatization. This method is based on a previous publication describing the chromatography of anilines, with the addition of a few modifications to eliminate some of the interferences found in urine[1].

There are several classes of pesticides that are metabolized or degraded to anilines, such as substituted ureas and carbamates. Anilines that are ingested or formed in the body by metabolism are generally excreted from the body in the urine. The analysis of anilines in the urine can provide an index of exposure to many of the pesticides in the environment. Pesticides, however, are not the only source of halogenated anilines in the environment and this index of exposure can only be used as a guide to possible sources. Also, there are several pesticides that can be metabolized to the same aniline. These include linuron, diuron and propanil, all of which are metabolized to 3,4-dichloroaniline when ingested.

The only existing method that could be found in the literature for the analysis of halogenated anilines in urine requires derivatization, silica gel cleanup and gas chromatography[2]. HPLC eliminates the need for derivatization and makes the cleanup easier. The existing method did not investigate the need for acid hydrolysis of the urine on biologically incorporated anilines. As suggested by Edgerton and Moseman[3], the use of urine in which the compounds of interest are biologically incorporated can give significantly different results when compared to urine which has simply been fortified in the laboratory. In this paper, we investigated the effect of acid hydrolysis on urine which contained biologically incorporated 3,4-dichloroaniline to determine the need for acid hydrolysis.

The anilines used in this study are by no means a complete list of anilines that can be found in urine. Other anilines can be added and the chromatography can be adjusted to provide the separation as needed[4]. The factors affecting separation will be discussed.

EXPERIMENTAL

A Tracor Model 995 isochromatographic pump with a Model CV-6-UHPa-HC Valco valve and a Model LC-2A electrochemical detector from Bioanalytical Systems[5,6] made up the chromatographic system. A 15-cm Zorbax® ODS column was used throughout the study. Two different mobile phases were used in this study because gradient elution cannot be used with the electrochemical detector. The anilines used in this study were divided into two groups depending on which mobile phase was required. The mobile phase used for aniline, *p*-chloroaniline and *p*-bromoaniline was a mixture of 80% 0.1 *M* phosphate buffer adjusted to a pH of 3.0 and 20% acetonitrile. The mobile phase mixture used for *m*-chloroaniline, *o*-chloroaniline and 3,4-dichloroaniline was 60% 0.15 *M* phosphate buffer adjusted to a pH of 2.1 and 40% acetonitrile. The electrochemical detector was operated at an oxidative potential of +1.1 V with a CP-W graphite paste electrode.

The standards used in this study were recrystallized or distilled under nitrogen from technical grade materials.

Cleanup of urine

The samples were hydrolyzed by adding 2 ml of 6 *N* HCl to 10 ml of urine in a screw cap culture tube. The tubes were then heated in a boiling water-bath for 1 h. The samples were removed from the water-bath, cooled to room temperature and washed four times with 5-ml portions of hexane which were discarded. A 3-ml volume of 10 *N* NaOH was added to neutralize the samples and the anilines were then extracted with three successive 4-ml portions of hexane. To return the anilines to an aqueous phase, the combined hexane fractions were successively extracted with 5, 3 and 2 ml of 5% phosphoric acid extracts were then combined, adjusted to final volume and injected directly into the liquid chromatograph.

RESULTS AND DISCUSSION

Urine samples fortified with known amounts of anilines were used to test the recovery of anilines from urine. Using simple acid–base–organic partitioning, the recoveries appeared to be good. However, as can be seen in Fig. 1, when urine containing biologically incorporated 3,4-dichloroaniline from rats fed linuron or diuron was analyzed and the results compared to the same urine which had been acid hydrolyzed, the amount of 3,4-dichloroaniline found in the hydrolyzed urine was at least ten times that found in the unhydrolyzed urine. As suggested by Edgerton and Moseman, this again points out the need for using biologically incorporated residues when developing methodology, especially methodology for polar residues. While it can be shown that the acid hydrolysis step increased the apparent level of 3,4-dichloroaniline, there is still no way of knowing if the hydrolysis releases all the anilines from the urine. Therefore, the levels found in urine should be considered a minimum level.

An attempt was made to develop a method for the analysis of anilines in soil, but even with acid hydrolysis the recovery from some fortified soils was as low as 50%.

The anilines appear to be stable to acid hydrolysis since the recovery of anilines

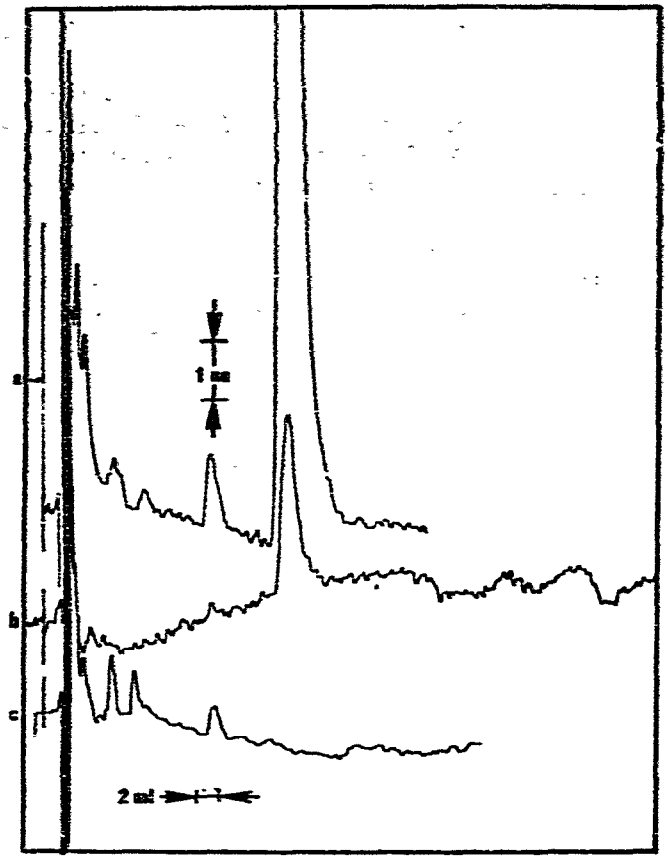


Fig. 1. Chromatograms of urine samples from rats fed linuron containing 3,4-dichloroaniline: a, acid hydrolyzed urine from rat fed linuron; b, unhydrolyzed urine from rat fed linuron; c, acid hydrolyzed control urine. na = nanoampère.

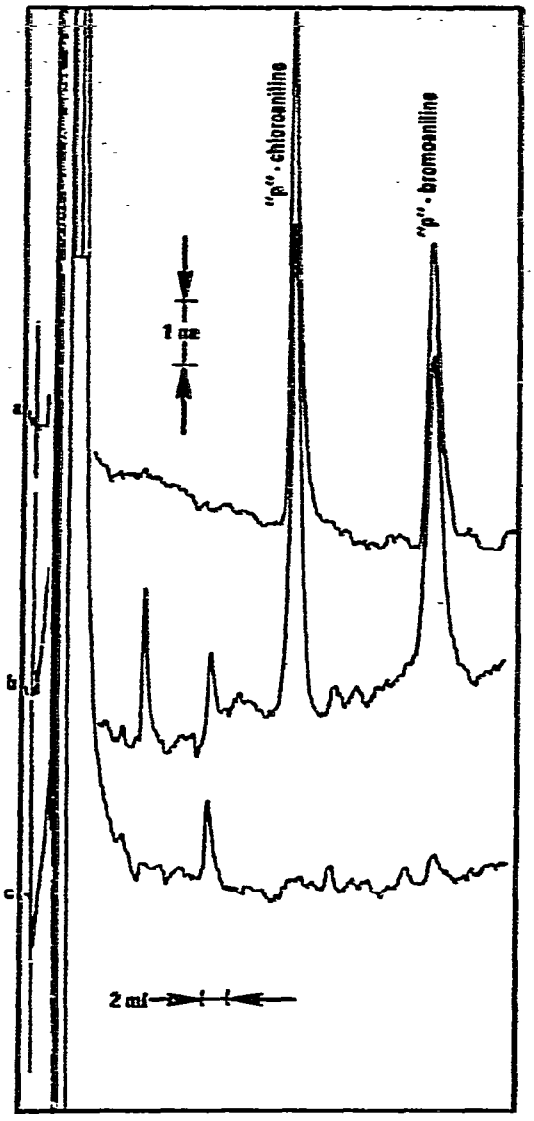
TABLE I

RECOVERY OF ANILINES FROM URINE

Compound	*Fortification level (ppb)*	*Recovery (%)*	
		Before hydrolysis	*After hydrolysis*
p-Chloroaniline	20	$\bar{x}_6$** 83	$\bar{x}_3$ 104
	50	$\bar{x}_6$ 86	
	100	$\bar{x}_6$ 81	$\bar{x}_5$ 93
p-Bromoaniline	20	$\bar{x}_6$ 98	ND* (interference)
	50	$\bar{x}_6$ 84	—
	100	$\bar{x}$ 86	$\bar{x}_3$ 94
m-Chloroaniline	20	$\bar{x}_2$ 82	
	50	$\bar{x}_2$ 80	$\bar{x}_3$ 100
o-Chloroaniline	20	$\bar{x}_2$ 80	
	50	$\bar{x}_2$ 80	$\bar{x}_3$ 81
3,4-Dichloroaniline	20	$\bar{x}_2$ 90	
	50	$\bar{x}_2$ 93	$\bar{x}_3$ 93

* ND = not determined.

** $\bar{x}_6$ = average of 6 determinations.



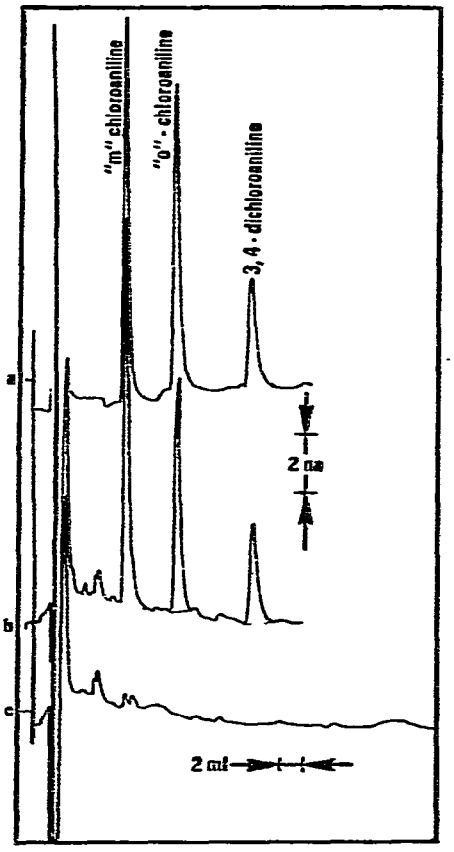


Fig. 2. Chromatograms of anilines on 15-cm C_{18} column with 80% pH 3 phosphate buffer and 20% acetonitrile: a, standard, 0.05 ppm; b, fortified urine sample, 0.05 ppm; c, control human urine.

Fig. 3. Chromatograms of anilines on 15-cm C_{18} column with 60% pH 2.1 phosphate buffer and 40% acetonitrile. Details as in Fig. 2.

from fortified urine samples with hydrolysis was greater than 80% for all the anilines tested. Table I shows the results of the recovery studies. Since the acid hydrolysis increases the interferences in the urine, the chromatography of one group had to be changed and an additional cleanup step had to be added to remove the interference. The hexane wash of the acid hydrolysate seemed to remove most of the interference. In this study, it was found that the older the urine sample was, the higher the level of

interference. No studies were made on the shelf life of the urine samples, but based on the increase in interference and the possibility of biological degradation of the anilines in the sample, it is believed the urine samples should be analyzed as soon as possible after collection.

The pH of the mobile phase used for *p*-chloroaniline and *p*-bromoaniline is higher than the pH reported in the previous paper[1]. This change in the pH was needed to separate the anilines from an interfering peak in hydrolyzed urine. Using the higher pH mobile phase the interfering peaks are completely separated as can be seen in Fig. 2. A chromatogram of the Group B anilines is shown in Fig. 3.

The mobile phase is circulated through the pumping system and detector 24 h/day, 7 days/week. This allows the detector to purge the mobile phase of any oxidizable compounds and eliminates the long warmup periods required for high sensitivity operation.

The limits of detection for this method will depend on the sample size and the noise level of the detector. In this study, with an injection volume of 70 μl and without concentration of the sample, levels below 5 ppb can be detected. Unsubstituted aniline cannot be detected using either of these mobile phases, since it is obscured by peaks eluting with the solvent front.

REFERENCES

1 E. M. Lores, D. W. Bristol and R. F. Moseman, *J. Chromatogr. Sci.*, 16 (1978) 358.
2 E. E. Bradway and T. Shafik, *J. Chromatogr. Sci.*, 15 (1977) 322.
3 T. R. Edgerton and R. F. Moseman, *J. Agr. Food Chem.*, 27 (1979) 197.
4 R. B. Sleight, *Chromatographia*, 6(1) (1973) 3.
5 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
6 P. T. Kissinger, *Anal. Chem.*, 49 (1977) 477.