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Note

Determination of chloroacetanilides and chloroanilines in sulphanilamides and waste water by high-performance liquid chromatography

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In the synthesis of sulphanilamide drugs the starting materials are acetanilide and chlorosulphonic acid from which acetylsulphanilyl chloride is prepared. The last named is converted into sulphanilamide drugs by treatment with various amine derivatives. Commercial chlorosulphonic acid contains up to 2% of pyrosulphuryl chloride as an impurity[1]. Chlorosulphonic acid sulphonates acetanilide, but pyrosulphuryl chloride acts as a chlorinating reagent to produce *p*-chloroacetanilide and to a lesser extent *o*-chloroacetanilide. Since chloroanilides and the corresponding chloroanilines are significantly soluble in water, they will be present in waste water discharges from factories producing sulphanilamides. The toxic nature of chloroanilines dictates that their concentrations in waste water have to be controlled, hence necessitating the use of a reliable analytical method for their determination.

The aim of the present work was to develop a method based on high-performance liquid chromatography (HPLC), that allowed separation and detection of nanogram quantities of *p*- and *o*-chloroacetanilide and of *p*- and *o*-chloroaniline in sulphanilamides and in waste-water produced in the manufacture of sulphanilamides. The separation of these compounds was accomplished on a C_{18}-bonded phase column, and monitoring was effected with a UV detector.

Sub-nanogram quantities of chloroanilines were detected by using the more sensitive electrochemical detector. Preconcentration on disposable Bond Elut columns packed with cyclohexyl-bonded silica made it possible also to detect the chloroacetanilides in sub-nanogram quantities.

EXPERIMENTAL

Equipment and materials

The equipment consisted of a Waters Model 6000 A pump, a Model 710 A WISP injector, a Pye-Unicam Model 4020 absorbance detector operated at 254 nm, and a Metrohm Model 656 electrochemical detector equipped with a Model EA 1096 detection cell (incorporating a glassy-carbon working electrode and an Ag/AgCl reference electrode) and a Model VA 641 potentiostat. The detector oxidation potential was +1.2 V, and the detector output was displayed on a Hewlett-Packard integrator Model 3390 A or on a Kipp & Zonen recorder Model BD 9.

The column used was a stainless-steel tube (25 cm × 4.6 mm I.D.) filled with

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LiChrosorb 10 RP-18 packed at the laboratory by using a downward tetrachloromethane slurry technique.

Bond Elut extraction columns (Analytichem International) contained 500 mg of cyclohexyl-bonded silica with a mean particle size of 40 μm.

The mobile phase consisted of acetonitrile–0.01 *M* ammonium carbamate (40:60) or methanol–0.05 *M* phosphate buffer of pH 7.0 (50:50). The injection volume was 20 μl and the flow-rate was 1 ml/min. All the solvents used were of HPLC quality (Merck). The chemicals were of reagent grade and used without further purification.

Sample preparation

Solids were dissolved in methanol. Acetylsulphanilyl chloride was hydrolyzed, after dissolution in methanol, by adding 2 *N* sodium hydroxide to a constant pH of 8.0. Solutions were neutralized (pH 6–8) by adding sodium hydroxide or hydrochloric acid. All the samples were passed through a 0.45-μm Millipore filter before injection.

Extraction of waste water

Bond Elut columns were activated with 5 ml of acetonitrile followed by 10 ml of distilled water. Sodium chloride (20 g) was dissolved in 100 ml of waste water, the solution was drawn through the pre-wetted column (with use of a vacuum line) at a flow-rate of *ca.* 2 ml/min, and the percolate was discarded. The column was washed with 10 ml of 20% sodium chloride solution in water and sucked dry, then 2 ml of 0.1 *N* hydrochloric acid–methanol (1:99) was applied as mobile phase, and the eluate was filtered through a 0.45-μm Millipore filter.

RESULTS AND DISCUSSION

The best isocratic separation of *p*-chloroacetanilide, *o*-chloroacetanilide, *p*-chloroaniline and *o*-chloroaniline was obtained with acetonitrile–0.01 *M* ammonium carbamate (40:60) as mobile phase. Other compositions based on phosphate buffer solutions of different concentrations and various pH values were tested, but these gave inferior results. Table I shows the capacity ratios for different mobile-phase compositions.

A typical chromatogram of hydrolyzed acetylsulphanilyl chloride, with acetonitrile–ammonium carbamate as mobile phase, is shown in Fig. 1. All four compounds can be detected in this sample. Some samples, however, contain compounds that interfere with the detection of *o*-chloroacetanilide and occasionally with that of *p*-chloroacetanilide. In these circumstances the mobile phase is changed to methanol–0.05 *M* phosphate buffer of pH 7.0 (50:50) (Table I). Fig. 2 shows a chromatogram of an acetylsulphanilylguanidine mother liquor analyzed under these conditions; in this instance *p*-chloroacetanilide elutes later from the column without interference. The retention time of *o*-chloroacetanilide can still be unsuitable for analytical purposes, but because the amount of this compound usually present is several times less than that of *p*-chloroacetanilide this is acceptable.

Otherwise the chloroacetanilides can be hydrolyzed to chloroanilines. The *o*- and *p*-chloroanilines elute close together in the methanol–phosphate system and can be difficult to distinguish from each other.

In waste water it is desirable to determine the chloroacetanilides and chlo-

TABLE I

CAPACITY RATIOS FOR DIFFERENT MOBILE PHASE COMPOSITIONS

Mobile phase	*pH*	*Compound*			
		o-Chloroacet-anilide	*p-Chloroacet-anilide*	*o-Chloro-aniline*	*p-Chloro-aniline*
0.02 *M* phosphate buffer–methanol (50:50)	3	1.5	3.7	3.0	3.6
	4	1.5	3.8	2.9	2.9
	5–8	1.4	3.3	2.7	2.5
0.05 *M* phosphate buffer–methanol (50:50)	3	1.4	3.3	2.7	2.7
	4	1.5	3.7	2.8	2.8
	5–8	1.6	3.8	3.1	2.8
0.05 *M* phosphate buffer–acetonitrile (58:42)	8	2.4	6.6	4.4	4.4
0.01 *M* ammonium carbamate–acetonitrile (60:40)	8.5	1.7	2.7	4.2	3.5

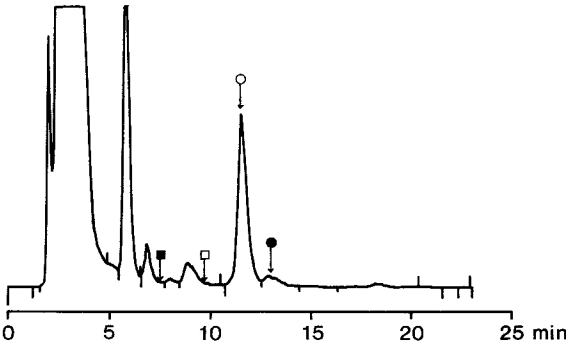

Fig. 1. Typical chromatogram of hydrolyzed acetylsulphanilyl chloride using acetonitrile–0.01 *M* ammonium carbamate (40:60) as mobile phase. UV detection. ○ = *p*-chloroaniline; ● = *o*-chloroaniline; □ = *p*-chloroacetanilide; ■ = *o*-chloroacetanilide.

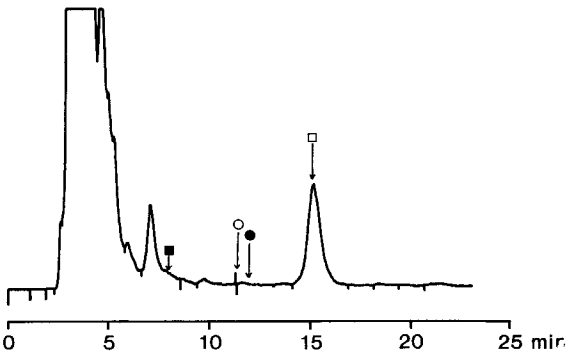

Fig. 2. Chromatogram of an acetylsulphanilylguanidine mother liquor using methanol–0.05 M phosphate buffer of pH 7.0 (50:50) as mobile phase. UV detector. ○ = *p*-chloroaniline; ● = *o*-chloroaniline; □ = *p*-chloroacetanilide; ■ = *o*-chloroacetanilide.

roanilines at the ppb level. This is not possible by using a UV detector. However, with an electrochemical detector, the chloroanilines can be detected at much lower concentrations. Fig. 3 shows a chromatogram of a waste-water sample containing 300 ppb* of *p*-chloroaniline and 45 ppb of *o*-chloroaniline. The mobile phase was methanol–0.05 *M* phosphate buffer of pH 7.0 (50:50). The oxidation potential was +1.2 V. Lores *et al.*[2,3] operated the detector at +1.1 V, but we have experienced an increase in the response of *o*-chloroaniline by one-third by increasing the potential. Unfortunately, at this high potential, the detector is not very selective.

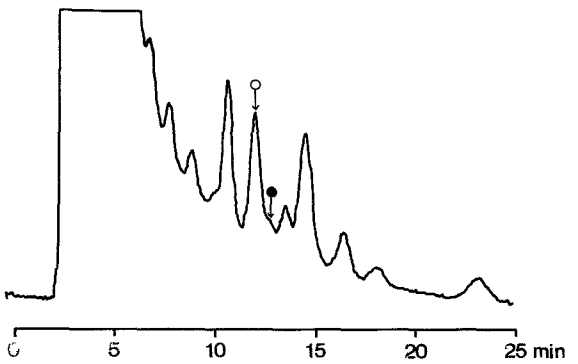

Fig. 3. Chromatogram of a waste-water sample. Mobile phase: methanol–0.05 *M* phosphate buffer of pH 7.0 (50:50). Electrochemical detection. ○ = *p*-chloroaniline; ● = *o*-chloroaniline.

The chloroacetanilides are not oxidized by the electrochemical detector and in order to detect these compounds at the ppb level a concentration procedure is necessary. Several extraction solvents have been evaluated, the most efficient being tributyl phosphate. A much simpler approach, however, was to isolate the chloroacetanilides on disposable extraction columns.

The chloroacetanilides and chloroanilines in 100 ml of an aqueous solution containing 300 ppb each of the four compounds were fully retained on a Bond Elut column packed with 500 mg of cyclohexyl-bonded silica. Consequently, other bonded phases were not evaluated. Table II shows the recoveries of the chloroacetanilides and chloroanilines obtained with different mobile phases. Chloroacetanilides can be eluted with 2 ml of the acetonitrile–ammonium carbamate mobile phase with good recovery. If also, the chloroanilines need to be concentrated, methanol–0.1 *N* hydrochloric acid (99:1) should be used as mobile phase. Better recoveries can be obtained by using additional mobile phase.

In testing the extraction procedure on waste water it was found that *p*-chloroaniline in certain samples was not completely retained on the extraction column. Addition of salt to the samples solved the problem. Table III shows the effect of adding different amounts of sodium chloride to a waste-water sample containing 275 ppb of *p*-chloroaniline. A concentration of 20% sodium chloride in the sample appears to be necessary to ensure complete extraction of *p*-chloroaniline.

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* Throughout this article the American billion (10^9) is meant.

TABLE II

RECOVERY OF CHLOROACETANILIDES AND CHLOROANILINES FROM 100-ml WATER SAMPLES RETAINED ON BOND ELUT CH-COLUMNS AND ELUTED WITH 2 ml OF DIFFERENT MOBILE PHASES

Mobile phase	*Recovery (%)*			
	o-Chloroacetanilide	*p-Chloroacetanilide*	*o-Chloroaniline*	*p-Chloroaniline*
0.05 *M* phosphate buffer (pH 7.0)–methanol (50:50)	92	16	62	78
0.01 *M* ammonium carbamate–acetonitrile (60:40)	99	92	69	82
Methanol	96	103	74	19
0.1 *N* hydrochloric acid–methanol (1:99)	107	105	83	99

TABLE III

RECOVERY OF P-CHLOROANILINE FROM 100-ml WASTE-WATER SAMPLES AS A RESULT OF SODIUM CHLORIDE ADDITION

NaCl added (g)	*p-Chloroaniline (%)*
0	39
10	71
20	102
30	102

Fig. 4 shows a chromatogram of a Bond Elut extract of a waste-water sample containing 275 ppb of *p*-chloroaniline and with 20 g of sodium chloride.

The detection limit for the four standard compounds (signal-to-noise ratio of 3) is in the range from 20 ng (chloroacetanilides) to 40 ng (chloroanilines) with the

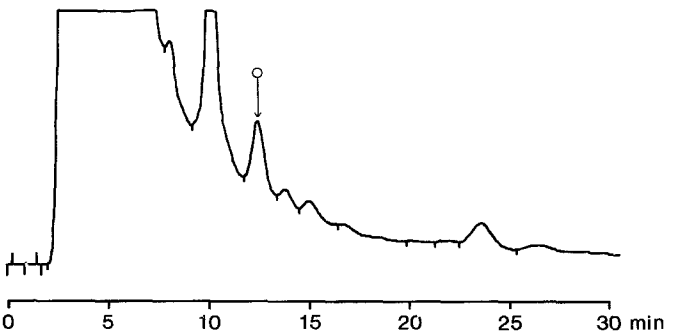

Fig. 4. Bond Elut extract of 100 ml of waste water containing 275 ppb of *p*-chloroaniline. Mobile phase: methanol–0.05 *M* phosphate buffer of pH 7.0 (50:50). UV detection. ○ = *p*-chloroaniline.

UV detector, and was *ca.* 0.8 ng for chloroaniline with the electrochemical detector (depending on the presence of interfering compounds).

The calibration graph for concentration of the four standard compounds *vs.* peak area was shown to be linear in the range 20 ng to 10 μg with the UV detector (correlation coef. $\geqslant 0.9991$) and in the range 0.5–20 ng of chloroaniline with the electrochemical detector (correlation coef. $\geqslant 0.9989$).

The reproducibility of the method was studied by preparing six samples of an acetylsulphanilylguanidine mother liquor, a hydrolyzed acetylsulphanilyl chloride sample and a waste water sample. The waste water sample was analyzed directly using the electrochemical detector and after concentration on Bond Elut columns using the UV detector. The results, shown in Table IV, exhibit good reproducibility (R.S.D. $\leqslant 5.7\%$).

TABLE IV
AMOUNTS OF CHLOROACETANILIDES AND CHLOROANILINES IN DIFFERENT SAMPLES

Sample	*Compound*	*ppm*	$\bar{x}$ *(ppm)*	*s (ppm)*	*R.S.D. (%)*
Acetylsulphanilylguanidine mother liquor	*p*-Chloroacetanilide	42.2–44.1–42.2 43.2–42.2–42.8	42.9	0.7	1.7
Hydrolyzed acetylsulphanilyl chloride	*p*-Chloroaniline	84.6–85.1–86.5 88.1–87.1–87.4	86.5	1.4	1.6
	o-Chloroaniline	12.2–10.3–11.7 11.8–11.8–11.6	11.6	0.7	5.7
Waste water	*p*-Chloroaniline	0.283–0.298 0.256–0.264 0.273–0.276	0.275	0.015	5.3
Waste water analyzed after concentration on Bond Elut columns	*p*-Chloroaniline	0.259–0.267 0.290–0.295 0.286–0.289	0.281	0.014	5.2

In conclusion, we have found the overall procedure described to be efficient for the determination of *o*- and *p*-chloroacetanilide and of *o*- and *p*-chloroaniline in sulphanilamides and waste water. The method is simple, sensitive and reproducible and is suitable for routine work, because sample handling and preparation is minimal.

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3 E. M. Lores, F. C. Meekins and R. F. Moseman, *J. Chromatogr.*, 188 (1980) 412.