Quantitative analysis of chlorophenothiazine by thin layer chromatography

Although several thin layer chromatographic techniques for the analysis of 10-substituted phenothiazines have been reported[1–4], those for 10-unsubstituted ones have not been established. We successfully applied the improved method of HASHIMOTO[5] to our study of the quantitative analysis of a mixture of 2- and 4-chlorophenothiazine whose determination by gas chromatography was unsuccessful.

About 120–160 μg of a mixture of 2- and 4-chlorophenothiazine in toluene solution was applied to the plate (200 × 50 mm) coated with silica gel* (0.25 mm thickness). Five holes of 2 mm diameter were engraved on the plate at regular intervals. Four holes were for the sample solution and the other was for the reference chromatogram. The sample solution was applied slowly by touching the bottom of each hole with a micropipet containing the solution and the holes were then plugged with silica gel. This procedure was more convenient than usual method[6–8] and gave good resolution.

The plate was developed with a mixture of petroleum ether and ether (3:2 by vol.) and each developed zone (R_F values of 2- and 4-chlorophenothiazine were approximately 0.7 and 0.5, respectively) was located by means of the reference chromatogram which had been sprayed with an 0.3 % solution of iodine in chloroform. Each zone was collected and eluted with absolute methanol. The eluate was determined spectrophotometrically at 256 mμ (λ_{max} of 2-chloro-isomer) and at 259 mμ (λ_{max} of 4-chloro-isomer), respectively. Blank corrections were carried out by using the eluate

TABLE I

RECOVERY OF 2- AND 4-CHLOROPHENOTHIAZINE FROM A KNOWN MIXTURE ON UNWASHED SILICA GEL

	Recovered μg						$\bar{X}$	R	%s	%Δ
2-Cl-isomer (61.5 μg applied)	58.2	60.5	57.5	59.5	61.7	61.2	59.77	4.2	2.47	—1.7
4-Cl-isomer (60.3 μg applied)	56.2	58.0	52.8	57.8	57.5	57.5	56.63	5.2	3.00	—3.7

TABLE II

RECOVERY OF 2- AND 4-CHLOROPHENOTHIAZINE FROM A KNOWN MIXTURE ON METHANOL-WASHED SILICA GEL

	Recovered μg					$\bar{X}$	R	%s	%Δ
2-Cl-isomer (122.2 μg applied)	121	121	117	121	119	119.8	4.0	1.31	—2.0
4-Cl-isomer (40.3 μg applied)	39.5	37.5	39.9	38.8	39.9	39.10	2.0	2.38	—3.0

$\bar{X}$ = mean (μg); R = range (μg); %s = relative standard deviation (%); %Δ = relative error (%).

* Silica gel with 10 % binder ($CaSO_4$) Wako gel B-10 (Wako Pure Chemical Industries, Ltd.).

from another plate for a blank. Silica gel washed with absolute methanol showed lower, and less deviation in, blank values than an unwashed one.

Recovery data are shown in Tables I and II. The method using washed silica gel gave excellent recovery and good relative standard deviation.

As mentioned above, our procedure of application of samples to the holes, has proved to be an excellent method.

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Production of ^{75}Se-selenocystine by neutron activation*

It has been noted that neutron activation can result in the labeling of selenium compounds[1]. This observation seemed surprising since it would be expected that the recoil energy, following neutron capture, would suffice to rupture carbon–selenium and selenium–selenium bonds. In view of the pronounced ability of mercaptans (and to a lesser extent disulfides) to act as free radical trapping agents[2], as well as the reported high scavenging activity of selenium[3], it seemed that the labeled molecules might be formed by the recombination of free radical fragments. For instance, it could be demonstrated that neutron activation of diphenylselenide yields both labeled diphenylselenide and labeled diphenyldiselenide[4]. In view of these observations, it appeared likely that neutron activation of symmetrical selenides and diselenides might be a useful method of preparing ^{75}selenium labeled compounds.

One hundred milligrams of selenocystine were irradiated for 30 h at a flux of $1 \cdot 10^{13}$ neutrons/cm^2/sec. If irradiation were uniform and there had been negligible self-shielding, the expected specific activity would be 5.8 μC/mg. Since the fractional abundance of ^{74}Se in nature is only 0.87 %, the specific activity could have been increased some 120-fold by utilizing the amino acid synthesized from pure ^{74}Se. The original amino acid was a light yellow, while the post-irradiation sample was distinctly red in color.

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