SPECTROPHOTOMETRIC DETERMINATION OF THIORIDAZINE WITH A SOLID-PHASE REACTOR OF Ce(IV) IN A CONTINUOUS-FLOW ASSEMBLY.

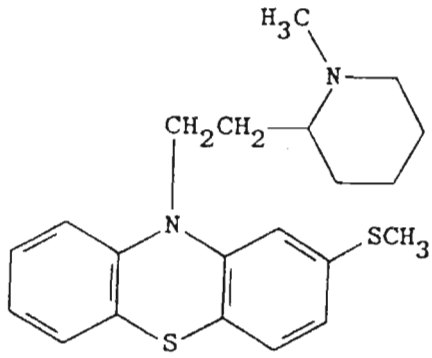
A. De Gregorio Alapont, J.V. García Mateo, I. Bernt**, J. Martínez Calatayud*
Departamento de Química. Colegio Universitario CEU. 46113. Moncada (Valencia). Spain.
*Departamento de Química Analítica. Universidad de Valencia. Valencia. Spain.

SUMMARY.- The spectrophotometric determination of thioridazine was carried out by a flow injection assembly provided with a solid-phase reactor with immobilized Ce(IV). The ceric oxidative reaction of thioridazine leads to a blue compound which was monitored spectrophotometrically at 636 nm. The calibration graph was linear over the range 5-140 μg ml^{-1} with a reproducibility of 2.2%; the sample throughput was 74 h^{-1}. The influence of foreign compounds was studied and the procedure was applied to thioridazine determination in pharmaceutical formulations.

Key-words.- Thioridazine, FIA, spectrophotometry.

*Author for correspondence
**Present address: Department of Chemistry, University of Erlangen, Erlangen, Germany.

Thioridazine is a drug of the antipsychotic phenothiazine group, one of the alkylpiperidine derivatives of the prototype phenothiazine. It was synthesized firstly in 1958 and, since then a certain number of papers has been published dealing with the clinical and chemical properties of the drug (1). Different analytical methods (mainly spectrophotometric and fluorimetric) have been proposed in batch procedures (1). The officially recommended procedure is based on the titration of thioridazine in glacial acetic acid and acetic anhydride against the solution of perchloric acid (2). A FIA procedure has been proposed for thioridazine determination (3); a different paper dealing with the use of solid-bed reactors as catalyst source in FIA (4) used the thioridazines as the test substance.



On the other hand, the use of solid-phase reactors (5)(6)(7)(8) (9) nesting into a FIA assembly present some advantages over its counterparts of dissolved reagents. Different strategies have been proposed for immobilization of reagents, namely; covalent bonding, adsorption, physical entrapment, etc. This paper is dealing with the immobilization of the strong oxidative reagent Ce(IV) by the type known as "natural" immobilization. The term "natural" immobilization can be used for low solubility reagents and with suitable chemical and mechanical stability to resist the routine work into a continuous-flow stream. The preparation of solid-phase reactors was carried out without using an auxiliary inert support .This "natural immobilization" of the ceric salts was published in a former paper (9) from this laboratory.

Reagents.- Aqueous stock solutions of thioridazine hydrochloride (Guinama) containing 100 mg ml^{-1} were prepared in distilled and deionized water. The solid-bed reactor was prepared from sodium arsenite (Panreac), nitric acid (Probus) and ammonium Ce(IV) nitrate (Merck) as described elsewere (9). Other reagents were: sucrose (Panreac), lactose (Panreac) and ethanol (Panreac).

Apparatus.- The proposed FIA assembly is depicted in Figure 1*a*. A model 5041 sample injector from Rheodyne and a Gilson Minipuls 2 pump were used. The determination of thioridazines was carried out by means of a Hewlet-Packard Model 8452A diode-array spectrophotometer at a wavelength of 636 nm. PTFE tube coils in the FI assembly were of 0.5 and 1.5 mm internal diameter for the carrier and the solid-phase reactor, respectively. Fluorimetric measurements were carried out by means of a Model LS 50 Perkin Elmer spectrofluorimeter.

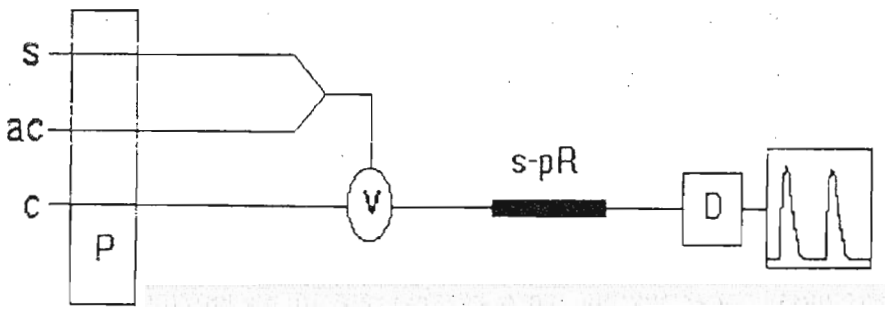


Figure 1a.- Flow-injection assembly proposed for the determination of thioridazine P, peristaltic pump; V, injection valve; D, detector; s-pR, solid-phase reactor; c, carrier; s, sample; and, ac, acid solution.

RESULTS AND DISCUSSION

Preliminary tests involved oxidizing thioridazine with a strongly acidic solution (0.5 M HNO_3, HCl, H_2SO_4 or H_3PO_4) of 0.001 M Ce(IV) in a manifold where the oxidant stream was merged with the drug solution (25 ppm) or, in blank runs, with distilled water. The fluorescence obtained (λ_{ex} = 254 nm, λ_{em} = 354 nm) was observed with the drug and blank solutions (in this case with lower values) and it was ascribed to Ce(III) produced in the reduction of Ce(IV) and released from the reactor.

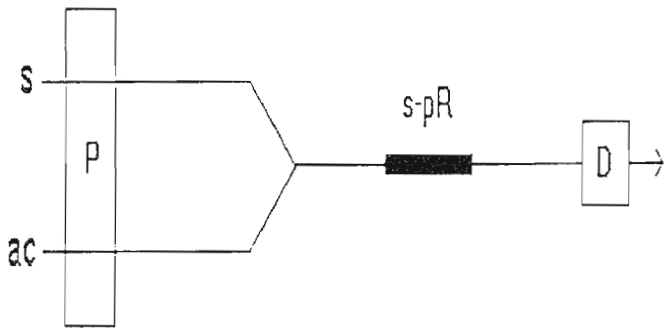


Figure 1b.- Flow-assembly for the study of the oxidation of thioridazine P, peristaltic pump; D, detector; s-pR, solid-phase reactor; s, sample; and, ac, acid solution.

Subsequent experiments were carried out in an assembly where the drug was merged with acid solution and the resulting mixture was passed through a solid-phase reactor (1.5 mm i.d. x 9.5 cm, particle size 150-200 μm) containing ceric arsenite on its way to the detector (Fig. 1*b*). The position of the excitation maximum was varied over the range 230–245 nm. The fluorescence emission was much higher than in the previous case, so emitted light was due not only to Ce(III) but also to the intermediate radical formed by oxidation of the drug. The fluorescence emitted by pure water was

low, but still significant. These observations are consistent with the effective oxidation of thioridazine and the release of Ce(III) from the reactor during the reaction or at a later stage (blank runs). The different intermediates produced in the drug oxidation (of variable colour depending on the particular medium used) resulted in poor reproducibility owing to the non-uniform release of Ce(III) and the absorption of emitted light by the oxidation products.

The FIA manifold of Fig. 1*a* was used to merge the sample (or blank) solution with the acid and drive the mixture to the injection valve for insertion into an acid carrier of variable composition or distilled water. The procedure used to condition the reactor (continuous circulation of the thioridazine solution for about 15 min) resulted in poor reproducibility for the same reasons as in the previous case. Therefore, spectrophotometric measurements were thought to result in smaller fluctuation and hence in better reproducibility.

The spectrophotometric detector was first tested in the manifold of Fig. 1*b* using the above-mentioned mineral acids at a 0.5 M concentration, and a reactor 1.5 mm i.d. x 10 cm packed with particles of size 150–200 μm. All the spectra observed exhibited an absorption maximum at 636 nm and peak heights were much smaller with phosphoric acid as the result of the reactor being partly blocked with cerous phosphate.

The highest absorbances were obtained in HCl, which was therefore chosen for subsequent experiments in the FIA assembly of Fig. 1*a*. The first few injections (0.5 M HCl) provided relatively low FIA signals. Hence the new reactor must be conditioned in order to increase the peak heights.

After the reaction variables and the dimensions of the solid-phase reactor were optimized, FIA variables were optimized by using a multivariate approach: the modified Simplex method [10, 11, 12]. The procedure was applied in a sequential manner: first

the ranges to be studied for each variable were established and then the optimized procedure was applied. Each vertex included not only peak height but also peak width and standard deviation (a minimum of 10 peaks per vertex). The results obtained were used to refine the ranges for the variables and the process was repeated. Finally, the seemingly best results were selected and the corresponding parameter values were used in many replicates in order to select those resulting in the best possible combination of sensitivity (peak height), throughput (peak width) and reproducibility (relative standarddeviation). The tested ranges were: flow-rate, 100-900 (arbitrary units); sample loop (in cm) 0-90; and, distance injector-reactor (in cm), 5-70. The optimized conditions were: flow-rate, 3.23 ml min^{-1}; sample volume, 433.8 μl; and,distance injector-detector, 50.0 cm.

Analytical applications

The calibration graph was linear over the range 5 - 140 $\mu g\ ml^{-1}$ thioridazine and fitted the equation $A = 0.0385 + 6.947 \times 10^{-3}\ C$ (where A is the absorbance and C the analyte concentration, in ppm). The day-to-day reproducibility was studied from five calibration curves constructed on different days. The relative standard deviation of the slope was 2.7% (n=5). The reproducibility of the determination was determined from replicate injections containing 50 ppm thioridazine (flow-cell 1 cm path-length). The relative standard deviation for 66 replicates was 2.2% and the throughput 74 samples/h.

The sensitivity and detection limit of the proposed method were substantially modified by increasing the path length of the flow-cell. In fact, replacing the spectrophotometer cell with another of 5 cm path length resulted in a linear calibration range from 1 to 25 ppm thioridazine ($A = 0.0338 + 0.040\ C$. $r = 0.996$).

The potential effects of compounds (excipients) frequently accompanying thioridazine hydrochloride in its formulations were studied by using solutions containing the drug (60 ppm) and variable concentrations of each interference. The percent errors in the determination of thioridazine produced by concentration of sucrose and lactose 1000 times higher than that of the analyte were 1.5% and 2.8% respectively. On the other hand, ethanol at the same concentration as the analyte gave rise to a relative error of 5.6%.

Finally, the thioridazine hydrochloride contents in three different pharmaceutical formulations were determined and compared with the manufacturers' certified contents to calculate the relative error, *viz.* 2.7% for Meleril (drops), 1.6% for Meleril 200 retard and 1.9% for Visergil (drops).

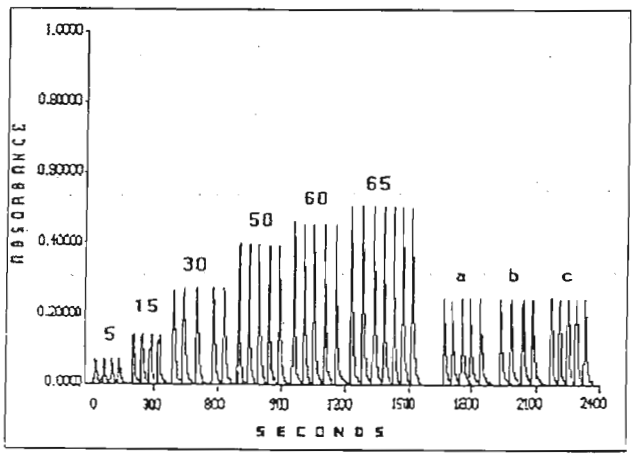


Fig.1c.- Calibration graph for thioridazine (ppm). a: Meleril;b: Meleril 200 retard;c: Visergil (30 ppm)

CONCLUSIONS

An spectrophotometric FIA procedure is proposed for the determination of thioridazine with application in the control analysis of pharmaceuticals. The method is based on the oxidation with cerium(IV) arsenite immobilized in a FIA manifold as solid bed reactor. The preparation of the reactor is easy and its lifetime is long enough to allow the processing of a reasonable number of samples.

REFERENCES

1. Analytical Profiles of Drug Substances. Ed. Klaus Florey; Ezzat M. Abdel-Moety and Khahid A. Al-Rashood, vol 18, pag. 459-525.

2. British Pharmacopoeia, Her Majesty Stationary Office, London 1992.

3. G. A. Rivas , A. Mellado Romero and J. Martínez Calatayud, **Anal. Chim. Acta**, in press.

4. J. L. Lopez Paz, J. V. Garcia Mateo and J. Martínez Calatayud, **Mikrochim. Acta.** , in press.

5. J. Martínez Calatayud, J.V. García Mateo, **Chem. Anal** (Warsaw) **38**, 1, 1993.

6. J. Martínez Calatayud, J.V. García Mateo,**Trends. Anal. Chem. 12**, 10, 1993.

7. J. Martínez Calatayud, J.V. García Mateo, **Anal. Chim. Acta, 274**, 275, 1993

8. J. Martínez Calatayud, J.V. García Mateo and L. Lahuerta Zamora, **Anal. Chim. Acta, 265**, 81,1992.

9. J. Martínez Calatayud, J.V. García Mateo, **Anal. Chim. Acta, 264**, 283, 1992

10. L. A. Yarbro and S.N. Deming, **Anal. Chim. Acta, 73**, 391, 1973.

11. S.L. Morgan and S.M. Deming, **Anal. Chem. 46**, 1170, 1974.

12. J.A. Nelder and R. Mead, **Comput. J., 7**, 808, 1965.

(Received January 18, 1996)
(Accepted June 10, 1996)