SHORT COMMUNICATIONS

DETERMINATION OF LEVAMISOLE HYDROCHLORIDE WITH HgI₂⁻ BY A TURBIDIMETRIC METHOD AND FLOW-INJECTION ANALYSIS

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(Received 30 October 1985. Revised 25 March 1986. Accepted 11 April 1986)

Summary—This paper is concerned with the use of ion-association compounds in the analysis of pharmaceutical samples by FIA. The usual extraction into an organic phase is avoided by using turbidimetric detection. Determination of levamisole with HgI₂⁻ has been developed as a practical example: the experimental variables were optimized by the modified simplex method. The calibration graph is linear over the levamisole concentration range 7–32 µg/ml. The reproducibility (rSD) and injection sample rate are 0.9% and 80/hr, respectively.

Few papers on flow-injection analysis (FIA) are concerned with turbidimetric detection, and so far only ammonia and sulphate ions have been determined in this way.

Krug et al. proposed using Nessler's reagent for turbidimetric detection of ammonia (in the 0.5–6 µg/ml range) in natural water samples. A protective agent was required in order to prevent gradual deposition of precipitate on the walls of the FIA system. Good precision and accuracy were achieved with a sampling rate of 120/hr.

Sulphate has been turbidimetrically determined with barium, at sampling-rates from 60/hr, to 250/hr. The widest application range was for sulphate concentrations from 5.0 to 200 µg/ml. Hemmings and Macdonald reported a procedure with 2-aminopyrimidine hydrochloride as reagent, using the merging-zones technique to save sample and reagent; 95 µl of both species are consumed in each determination, in the 0–10 µg/ml sulphate range, and the sampling-rate is up to 60/hr.

Liquid-liquid distribution of ion-association compounds and their photometric detection is a broadly used procedure in drug analysis: it is a sensitive and precise technique for the determination of basic drugs. Since the initial work of Karlberg and Thelander on determination of caffeine in acetylsalicylic acid preparations, with sodium laurylsulphate interference eliminated by addition of tetrphenylammonium ion (which forms an ion-pair with the laurylsulphate and transfers it to the organic phase, where it does not contribute to the analyte absorbance at 275 nm), some applications of FIA automated solvent extraction and ion-association complex formation to drug evaluation have appeared in the literature. They include determination of codeine with picrate, procyclidine hydrochloride, also with picrate, and anionic and cationic surfactants in pharmaceutical formulations. Steroid and bile acid sulphates have been determined in clinical analysis with lucigenin.

This paper is concerned with turbidimetric detection in FIA procedures based on ion-association compounds, and deals with evaluation of levamisole with tetraiodomercurate(II) as precipitating reagent. The procedure was optimized by the modified simplex method (MSM), the parameters concerned being sample volume, reaction coil-length, flow-rate, pH and reagent concentration.

Levamisole hydrochloride is the laevio-isomer of tetramisole hydrochloride; both are anthelmintic drugs, but the former has fewer side-effects. Holbrook and Scales have determined tetramisole in animal tissue extract polarographically, and Mourot et al. have evaluated it by HPLC in the routine analysis of veterinary anthelmintics, but both methods require mg quantities of the drug. Some liquid extraction–colorimetry methods have been proposed for evaluation of tetramisole in µg amounts, with various dyestuffs. Sodium nitroprusside and cobalt thiocyanate have also been proposed as reagents.

No pharmacopoeial method is given in the BP or USP for determination of the two drugs.

EXPERIMENTAL

Reagents

Aqueous solutions of levamisole hydrochloride. The solid (donated by Quimica Farmaceutica Bayer) was found, by non-aqueous potentiometric titration with perchloric acid in acetic acid medium, to be 100.7 ± 0.3% pure (5 replicates). Channing's solution. Mercuric iodide (1.00 g) and potassium iodide (0.80 g) were dissolved, mixed and diluted to 100 ml with demineralized water.

Buffer solutions. Made with citric acid and disodium hydrogen phosphate, at 0.5M ionic strength and adjusted to the desired pH.
**Carrier stream.** A mixture of 10 ml of buffer solution and \( V \) ml of Channing's solution was made up to 50 ml with demineralized water.

Other reagents were of analytical grade.

**FIA assembly and procedure**

The sample solution was injected into the carrier stream and the turbidity measured spectrophotometrically with a Coleman 55 (Perkin-Elmer) instrument provided with an 18-µl flow-cell (Hellma) and a Unicam 45 A-R (Pye Unicam) recorder. The Tecator 5020 apparatus, sample injector and pumps were used and the reaction-coil was a 0.5-mm bore Teflon tube.

**RESULTS AND DISCUSSION**

A qualitative study of formation of ion-association compounds of levamisole was done by mixing 1 ml of \( 4.2 \times 10^{-4}M \) levamisole solution, 1 ml of \( 1.20 \times 10^{-3}M \) dyestuff or \( 2.64 \times 10^{-5}M \) inorganic anion and 1 ml of buffer. No precipitate was formed with Bromocresol Purple or Green, Thymol Blue, Bromophenol Blue, Methylthymol Blue, Phenol Red and Arsenazo B, but \( \text{HgI}_2^- \), \( \text{CdI}_2^- \) and \( \text{BiI}_4^- \) gave yellow, white and orange precipitates respectively.

Spectra from 400 to 750 nm for suspensions obtained from \( 5.3 \times 10^{-5}M \) levamisole plus \( 2.64 \times 10^{-3}M \) \( \text{HgI}_2^- \) or \( \text{BiI}_4^- \) solutions were recorded in order to choose the most suitable counter-ion for levamisole determination (no precipitate was obtained with levamisole and \( \text{CdI}_2^- \) at these concentrations). The influence of levamisole concentration was also tested.

Figure 1a shows the spectra measured against demineralized water immediately after production of the turbidities. The absorption peak at 430 nm for the levamisole-\( \text{HgI}_2^- \) system is due to the ion-association compound, since no absorption is observed with the clear solution. For the levamisole-\( \text{BiI}_4^- \) system no absorption peak was observed, so absorbance measurements were made at 700 nm, where the reagent does not absorb.

The calibration graph is steeper for the \( \text{HgI}_2^- \) system (Fig. 1b) so this is preferred for practical use, although the limit of detection is lower for the \( \text{BiI}_4^- \) system.

Spectra over the range 400–500 nm recorded at 30-sec intervals show a decrease in absorbance with time; the stability is not improved by addition of protective colloids, such as sucrose, glucose and starch, and this prevents use of this reaction in a batch procedure.

The influence of pH was tested by adding hydrochloric acid or sodium hydroxide solution to a \( 5.6 \times 10^{-4}M \) levamisole/\( 2.11 \times 10^{-3}M \) \( \text{HgI}_2^- \) mixture and recording the absorbance at 430 nm; the best pH range was found to be 3.30–7.65. Various buffer solutions were tested and the citric acid/phosphate system was selected.

The stoichiometry of the product was established by conductometric titration of 55 ml of \( 8.1 \times 10^{-4}M \) \( \text{HgI}_2^- \) or \( 3.20 \times 10^{-4}M \) levamisole with \( 2.00 \times 10^{-2}M \) levamisole or \( 2.20 \times 10^{-3}M \) \( \text{HgI}_2^- \) respectively (see Fig. 2) and found to be 2:1 levamisole:\( \text{HgI}_2^- \). It is a relatively weak compound, because precipitation is not clearly observed until there is a 20-fold molar ratio of \( \text{HgI}_2^- \) to levamisole and the maximum

![Fig. 1. (a) Spectra of 7.0 \times 10^{-3}M; levamisole solutions: 1, with 2.6 \times 10^{-3}M \text{HgI}_2^-; 2, blank solution for 1; 3, with 2.64 \times 10^{-3}M \text{BiI}_4^-; 4, blank solution for 3. (b) Calibration curve: \( \lambda = 430 \) nm, \( \lambda = 700 \) nm.](image)

![Fig. 2. Conductometric titrations: 1, titrant levamisole; 2, titrant \text{HgI}_2^-.](image)
absorbance is obtained at > 50-fold molar concentration ratio.

**MSM of optimization**

The range of FIA variables to be studied was provided by the results of the preliminary spectrophotometric work. The peak-height was the parameter to be optimized. The range of variables is shown in Table 1.

Once a stable chart-recorder base-line had been obtained, a sample was injected, the reaction took place and the resulting peak was collected and the absorbance value at 430 nm read. This was repeated until an rsd ≤ 1% was obtained for the peak-height (four or five repetitions usually sufficed).

The program of the MSM for this work was operated with six vertices, and was written on the basis of references 24–26. The initial simplex was chosen according to Yarbro and Deming with a side-length of 1 and the vertex at the origin of the co-ordinates. The region of the variables was normalized by the modification proposed by Morgan and Deming. The optimal conditions obtained for 1.0 × 10⁻⁴ M levamisole were: pH 5.00, flow-rate 3.14 ml/min, reaction-coil length 77 cm, sample size 200 µl and reagent concentration 5.8 × 10⁻³ M.

The influence of ionic strength on the peak-height is constant over the range 0.07–0.12 M (Fig. 3.).

The reproducibility of the analysis was tested by injecting into the reagent stream 40 samples of 9.1 × 10⁻³ M levamisole; the rsd was 0.9% (similar to that of the method of Sane et al.). The sample injection rate obtainable was 80/hr.

No cleaning solution was used for the manifold tubing, but of those tested 6 M hydrochloric acid gave good results.

The levamisole content of Nemanthel was determined (veterinary drug, declared formulation: 10 g of levamisole, 0.1 g of clorferamine maleate, and excipient to give 100 g total weight of sample). A 0.5-g portion of previously powdered sample was dissolved and diluted accurately to 250 ml with demineralized water, and further diluted ten-fold (5 ml to 50 ml) and 200 µl were directly injected into the carrier; the result obtained was 11.8 ± 0.2% (average value of three replicates). This sample was checked by the method of Sane et al. with Bromocresol Purple, the average of 3 replicates being 12.0 ± 0.2%.

**REFERENCES**