Determination of Chlordiazepoxide by Zinc or Cadmium Reduction in a Continuous System Followed by Atomic Absorption Spectrometric Detection

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The analytical possibilities of using simple unsegmented configurations including redox columns for the selective determination of organic compounds with atomic absorption spectrometric detection are discussed. A method based on reduction with cadmium or zinc was developed for the determination of chlordiazepoxide in pharmaceutical preparations. This drug can be determined in the range 2.0–25 μg ml^{-1} with a relative standard deviation between 1.1 and 2.8% and a sampling frequency of 150 h^{-1}. The method is selective towards the reduction of the *N*-oxide group and permits the determination of chlordiazepoxide in the presence of other 1,4-benzodiazepines.

Keywords: *Atomic absorption spectrometry; flow injection; chlordiazepoxide determination; metal reducing column*

Chlordiazepoxide is a major sedative - hypnotic drug widely employed as a tranquilliser and antidepressant. Like other 1,4-benzodiazepines, it has been extensively studied.[1,2] Several techniques, including spectrophotometry,[3] spectrofluorimetry,[4] polarography,[5,6] thin-layer chromatography,[7] high-performance liquid chromatography (HPLC),[8,9] gas chromatography (GC)[10] and plasma chromatography[11] have been reported for its determination. The US Pharmacopeia[12] and the European Pharmacopoeia[13] propose a spectrophotometric method based on the acid hydrolysis of the drug to its corresponding aminobenzophenone, followed by formation of a violet complex by diazotisation and coupling with *N*-1-naphthylethylenediamine. In addition, GC analysis is further complicated by the thermal instability of some of these compounds, particularly chlordiazepoxide, and HPLC analysis often requires a carefully selected mobile phase and is very expensive.

The high selectivity and sensitivity offered by atomic absorption spectrometry (AAS) has been exploited for the indirect determination of various organic pharmaceutical compounds by manual procedures.[14,15] The only AAS method for the determination of two benzodiazepines (bromazepam and flurazepam) reported so far is based on the extraction of these drugs as ion pairs into isobutyl methyl ketone.

There has been increasing interest in automated methods of analysis in recent years.[16–18] Different types of solid reactors have been employed in conjunction with unsegmented-flow configurations as ion-exchange columns, enzyme reactors and redox reactors. The last type have been used for the speciation of nitrite and nitrate by the modified Griess reaction using cadmium and copper-coated cadmium columns.[19,20] The analytical applications of unstable oxidising and reducing agents in aqueous solutions in flow analysis were recently reviewed by den Boef.[21]

This paper reports an indirect method using flame AAS detection for the determination of chlordiazepoxide (Librium) in pharmaceutical preparations. It is based on the selective reduction of the *N*-oxide group by using reducing columns coupled on-line to a flow injection system. A detailed study of the different analytical possibilities of various reducing columns (some of which were originally designed for this work), which can be located either inside or outside the loop of the valve, was made. Other benzodiazepines are not reduced by these systems.

Experimental

Instruments and Apparatus

A Perkin-Elmer 380 atomic absorption spectrometer equipped with suitable hollow-cathode lamps (zinc or cadmium) and an adjustable nebuliser was used. The instrument was operated with deuterium-arc background correction and the air - acetylene flame was adjusted according to the recommendations of the manufacturer. The spectrometer output was connected to a Radiometer REC-80 Servograph recorder. The peristaltic pump was a Gilson Minipuls-2, furnished with poly(vinyl chloride) pump tubing. The injector consisted of a rotary valve (Tecator Model L 100-1) to which a loop of the required volume was fitted; PTFE tubing (0.5 mm i.d.) and a selection valve (Rheodyne Model 5041) were also used. Different laboratory-made columns were employed for reduction purposes.

Reagents

The benzodiazepines were obtained from Sigma and Roche in both pure and tablet forms. The ethanol solutions of the drugs were protected from light during the analyses. A stock solution of chlordiazepoxide hydrochloride (1.000 g l^{-1}) was prepared in absolute ethanol and was stable for several weeks. Less concentrated solutions were obtained by dilution of this stock solution with distilled water.

Redox Columns

The reduction columns were made by packing a glass capillary (4.5 or 8.5 cm × 1.8 mm i.d.) with cadmium or zinc granules (Merck) of medium size (grain diameter 0.5–1.2 mm). Copper-coated cadmium columns (4.5 or 8.5 cm long) were prepared in a similar manner with cadmium granules that had been previously coated with copper by passing a 0.1% copper(II) sulphate - 0.1 M ethylenediaminetetraacetic acid solution over them. Amalgamated zinc columns were prepared by packing the capillary (4.5 cm or 8.5 cm long) with zinc granules and then passing 2% nitric acid - 0.25 M mercury nitrate solution through it.

Pharmaceutical Preparations

Each commercial sample (5–15 tablets) was placed in a mortar and ground to a fine powder. An amount of powder equivalent to 15–20 mg of pure chlordiazepoxide was placed in a 100-ml vessel, 50 ml of ethanol were added and the mixture was shaken electromagnetically with heating at 40–50 °C for 1 h. After cooling the solution to room temperature, it was filtered and the residue was washed with water; subsequently, the filtrate was diluted to volume with water in a 250-ml calibrated flask. For continuous-flow analyses, different aliquots of the sample solution (3.0–4.5 ml) were placed in 25-ml calibrated flasks and diluted to volume with distilled water after adjusting the pH to 3.5–5.0 with 10^{-2} M hydrochloric acid.

Procedure

The continuous-flow manifolds used are depicted in Fig. 1. In the first configuration [Fig. 1(*a*)] the reducing column was located between the injection valve and the detector and in the second [Fig. 1(*b*)] it was placed in the loop of the injection valve. In both instances, the sample solution containing 6×10^{-6}–7.5×10^{-5} M chlordiazepoxide at pH 3.5–5.0 was injected (200 µl) into a carrier solution (distilled water). First, a water blank (of the same pH as the sample) was injected and a peak due to the small amount of metal (cadmium or zinc) dissolved from the column in acidic media was obtained. Second, the sample was injected and a peak due to the redox reaction (reduction of the *N*-oxide group and oxidation of the metal) was obtained. The difference between the two peaks gave the amount of drug injected, which was proportional to the chlordiazepoxide concentration in the sample. All reagents and instrumentation were kept at room temperature throughout the experiments.

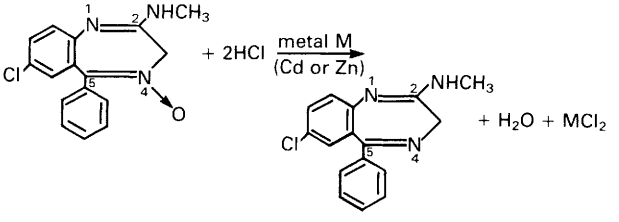
Results and Discussion

Only two major 1,4-benzodiazepines have structures divergent from their generic type, namely medazepam and chlordiazepoxide. The *N*-oxide group is an unusual structural feature in biological systems; the only benzodiazepine which includes it is chlordiazepoxide. The reduction of this group by metals does not appear to have been investigated so far.

Reduction Conditions and Products

The reduction of chlordiazepoxide with cadmium (pure and copper-coated) and zinc (pure and amalgamated) columns was examined by using the manifold depicted in Fig. 1(*a*). The reaction only developed in an acidic medium (hydrochloric or sulphuric acid); in addition, the time over which it was allowed to proceed was irrelevant as it was almost instantaneous. On the other hand, assays carried out with the copper-coated cadmium and amalgamated zinc columns showed the metals involved in the reaction to be cadmium and zinc, as no copper or mercury was detected with the corresponding lamps in AAS on injecting chlordiazepoxide into the system; only cadmium or zinc was detected, depending on the column used, at concentrations analogous to those found with the pure metal counterparts.

As chlordiazepoxide includes several reducible groups we recorded an infrared (IR) spectrum. For this purpose, a 1 g l^{-1} chlordiazepoxide solution of pH 3.5 (adjusted with hydrochloric acid) was passed continuously through a zinc or cadmium reduction column for 30 min. Aliquots of the reduction products from both columns were extracted with toluene or diethyl ether and their IR spectra were recorded after evaporating the organic solvents. The IR spectra obtained for the reduction products from both columns extracted into different organic solvents were very similar. They showed no N–O stretching band at 1240 cm^{-1}, in contrast to the spectrum for pure chlordiazepoxide. These results indicate that the N–O group loses the oxygen, the reaction involving the uptake of two electrons and two protons according to the following equation:



This is consistent with literature reports which state that of the three reducible functional groups in chlordiazepoxide (N^4–O, N^1=C^2 and C^5=N^4), the first is the easiest to reduce.[22,23] In addition, other benzodiazepines lacking the N^4–O group but containing the other two (*e.g.*, medazepam and diazepam) are not reduced under the same experimental conditions as chlordiazepoxide [see manifold in Fig. 1(*a*)].

Experimental Conditions

The pH was the only variable that affected the redox reaction while the flow injection variables were kept constant. The pH of the sample (10 µg ml^{-1} of chlordiazepoxide) and the blank (water) was varied in the range 3.0–8.5. The absorbance difference remained constant over similar pH ranges for the different redox columns (pure and copper-coated cadmium, pure and amalgamated zinc), *viz.*, 3.5–5.0. Below pH 3.5, the blank signal fell outside the linear range of the instrument owing to the dissolution of the metal in the acidic medium. The pH of all these solutions was adjusted with dilute hydrochloric acid or ammonia.

The influence of the temperature was studied in the range 10–80 °C by thermostating the sample and blank solutions and the redox reactor. Temperature had no effect on the redox reaction. Therefore, it was decided to work at room temperature for convenience.

The effect of the injection volume on the peak height at a constant flow-rate and chlordiazepoxide concentration (10 µg ml^{-1}) was studied by using the two manifolds depicted in Fig. 1. The absorbance increased as the amount of sample injected into the water carrier increased. Therefore, both the blank and the sample signal were dependent on the plug width, but the difference between them remained constant for 170–350 µl. As the injection of larger volumes increased the peak width (thus reducing the sample throughput), a volume of 200 µl was chosen for both manifolds.

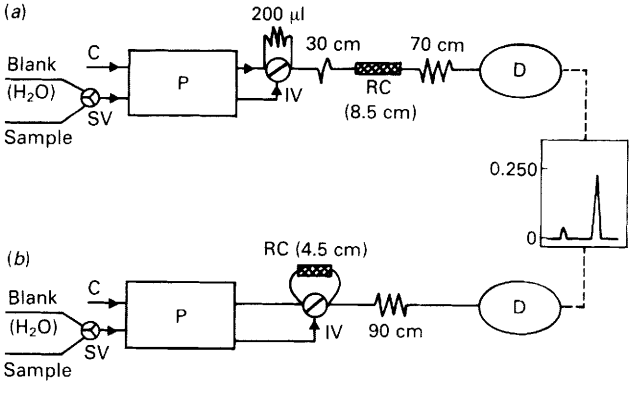


Fig. 1. Flow injection configurations used for the determination of chlordiazepoxide. C, carrier (water); P, peristaltic pump; SV, selection valve; IV, injection valve; RC, redox column; and D, detector

Table 1. Characteristic parameters of the calibration graphs and analytical features of the determination of chlordiazepoxide

Column type	Regression equation*	Correlation coefficient	Detection limit/ μg ml^{-1}	RSD, %
Zn (8.5 cm)	$A = -0.003 + 11.5 \times 10^{-3}c$	0.998	1.3	2.8
Zn (4.5 cm)	$A = -0.001 + 9.5 \times 10^{-3}c$	0.999	1.1	2.6
Zn - Hg (8.5 cm)	$A = 0.001 + 9.7 \times 10^{-3}c$	0.999	1.2	1.1
Zn - Hg (4.5 cm)	$A = 0.003 + 8.2 \times 10^{-3}c$	0.997	1.4	2.1
Cd (8.5 cm)	$A = 0.002 + 9.5 \times 10^{-3}c$	0.999	0.9	2.5
Cd (4.5 cm)	$A = -0.001 + 9.7 \times 10^{-3}c$	0.999	1.0	1.3
Cd - Cu (8.5 cm)	$A = 0.001 + 9.7 \times 10^{-3}c$	1.000	1.0	2.2
Cd - Cu (4.5 cm)	$A = 0.001 + 9.8 \times 10^{-3}c$	0.996	0.9	1.9

* Absorbance (A) *versus* chlordiazepoxide concentration (c, μg ml^{-1}).

Table 2. Determination of chlordiazepoxide in dosage forms

Sample*	Nominal content per tablet, %	Found, % Zinc column	Found, % Cadmium column
Huberplex 5	3.7	3.6 ± 0.04	3.6 ± 0.04
Huberplex 10	7.5	7.4 ± 0.05	7.5 ± 0.08
Huberplex 25	18.7	18.7 ± 0.1	18.6 ± 0.2
Librax	1.5	1.5 ± 0.02	1.5 ± 0.01
Librium 5	5.8	6.0 ± 0.1	5.9 ± 0.1
Librium 10	5.8	6.0 ± 0.1	6.0 ± 0.1

* Huberplex tablets (Hubber, Barcelona, Spain) labelled to contain 5, 10 or 25 mg of chlordiazepoxide each. Librax tablets (Roche, Madrid, Spain) labelled to contain 5 mg of chlordiazepoxide and 2.5 mg of clidinium bromide each. Librium tablets (Roche) labelled to contain 5 or 10 mg of chlordiazepoxide each.

Table 3. Recovery of chlordiazepoxide added to pharmaceutical preparations

Sample	Aliquot/ ml	Content/ μg ml^{-1}	Added/ μg ml^{-1}	Recovery, %
Huberplex 25	0.8	2.5	5.0	102.0
	0.8	2.5	10.0	97.6
	1.6	5.0	5.0	100.0
	1.6	5.0	10.0	101.3
	3.2	10.0	5.0	99.3
	3.2	10.0	10.0	100.0
Librax 5	1.0	2.3	5.0	97.3
	1.0	2.3	10.0	99.2
	2.0	4.6	5.0	101.7
	2.0	4.6	10.0	100.0
	4.0	9.2	5.0	102.1
	4.0	9.2	10.0	100.5
Librium 10	1.1	2.8	5.0	100.0
	1.1	2.8	10.0	99.2
	2.2	5.6	5.0	102.1
	2.2	5.6	10.0	100.6
	4.4	11.2	5.0	101.3
	4.4	11.2	10.0	98.7

The influence of the flow-rate of the water carrier on the peak height was examined in the range 1–5 ml min^{-1}. The signal increased with increasing flow-rate and was constant above 3.0 ml min^{-1}. A flow-rate of 3.5 ml min^{-1} was chosen.

The length of the redox columns did not affect the extent of development of the reaction as the reduction of chlordiazepoxide was almost instantaneous; therefore, columns of 1.8 mm i.d. and of length 8.5 cm outside the injection loop and 4.5 cm inside it were used. Changes in the column compactness caused by the uninterrupted flow in the same direction had no effect on the chemical reaction or the flow-injection system. The column lifetime was at least 2 months. The copper-coated cadmium and amalgamated zinc columns lost their coated layers in a few days; however, they required no reactivation because, as stated above, the coating elements were not electroactive and so did not take part in the reaction. The lifetimes of the 8.5-cm columns were typically double those of the 4.5-cm columns.

Determination of Chlordiazepoxide

By using the manifolds depicted in Fig. 1, several linear calibration graphs were obtained for chlordiazepoxide with the different columns. Table 1 lists the characteristic parameters of these graphs and the analytical features of the determination of chlordiazepoxide in the range 2.0–25 μg ml^{-1}. The detection limit was calculated as three times the standard deviation of the peak height for 30 determinations of the same blank. The precision of the method was checked on 11 samples each containing 10 μg ml^{-1} of chlordiazepoxide. The sensitivities (slopes of the calibration graphs) were similar for the zinc and cadmium columns, taking into account that although the relative atomic mass of the latter is about twice that of the former, the direct sensitivity of the element by AAS is approximately double for the former. The sampling frequency achieved was 150–200 h^{-1}.

To test the selectivity of the method towards the reduction of the *N*-oxide group, other 1,4-benzodiazepines were assayed using the manifolds depicted in Fig. 1 with cadmium and zinc redox columns. The benzodiazepines assayed were diazepam, oxazepam, medazepam, flurazepam, bromazepam, lorazepam and nitrazepam. Foreign species were added at a maximum level of 1 mg ml^{-1} per 10 μg ml^{-1} of chlordiazepoxide. There was no interference from any of the substances studied, except nitrazepam, which was tolerated at concentrations only five times those of chlordiazepoxide. This interference can be explained on the basis of reduction of the nitro group to the corresponding amine.[24]

Applications

The proposed method was applied to the determination of chlordiazepoxide in commercial pharmaceutical preparations by using the manifold in Fig. 1(*a*). The results are given in Table 2. A recovery study was carried out with the zinc column (8.5 cm × 1.8 mm i.d.) by adding different amounts (125 or 250 μg) of chlordiazepoxide to each pharmaceutical preparation and diluting to volume with distilled water in a 25-ml calibrated flask after adjusting the pH to 3.5–5.0. The recoveries obtained are summarised in Table 3. They ranged from 97.3 to 102.1% with relative standard deviations between 1.5 and 2.8%.

Conclusions

The analytical possibilities of various cadmium (pure and copper-coated) and zinc (pure and amalgamated) reducing columns of various dimensions located inside or outside the loop of the injection valve in continuous systems coupled on-line to an AAS detector have been studied for the first time. The results obtained allow the following conclusions to be drawn: there is no clear gain in using coated cadmium or zinc beads; the length of the column has no effect when the redox reaction is instantaneous, although their lifetimes are proportional to their dimensions; and the reproducibility of the signals is not affected by changes in the column compactness (despite the undirectional flow used).

The proposed systems are simple, rapid, accurate and specific towards the reduction of the *N*-oxide group of chlordiazepoxide and they could be applied to the determination of this drug in the presence of other 1,4-benzodiazepines in routine analyses of various pharmaceutical preparations. Chlordiazepoxide hydrochloride is metabolised in man to form two major metabolites, *N*-desmethylchlordiazepoxide and the lactam (demoxepam), both of which bear the *N*-oxide group; the proposed method could be applied to the determination of chlordiazepoxide and its metabolites in biological fluids.

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