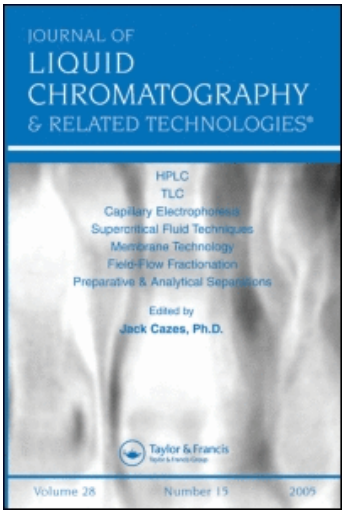


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# QUALITY CONTROL OF PHARMACEUTICAL FORMULATIONS OF NEUROLEPTIC DRUGS BY CAPILLARY ZONE ELECTROPHORESIS

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## ABSTRACT

Classical (haloperidol and chlorpromazine) and atypical neuroleptics (olanzapine, clozapine, loxapine, and risperidone) were quantified in pharmaceutical formulations (tablets, capsules, and oral solutions) by capillary zone electrophoresis. The simple sample pretreatment consists of a one-step extraction, filtration, and dilution. The electrophoretic conditions were as follows: uncoated fused-silica capillary (28.3 cm total length, 22.0 cm effective length, 50 μm I.D.), phosphate buffer (pH 2.5, ionic strength 35 mmol/L). The separation voltage (15 kV) results in a current lower than 50 μA. UV detection was performed at 214 nm. Calibration curves were linear in the 5-50 μg/mL range for all tested drugs ($r$ better than 0.999). The repeatability (or intra-day precision), expressed by the relative standard deviation, was better than 2% (6 measurements). The accuracy, resulting from recovery studies, was between 98 and 105 %. The amount of drug found agreed with the declared contents within the limits specified by U.S.P. (1995).

## INTRODUCTION

In the last years the introduction of new chemical compounds with neuroleptic properties into the clinical practice seems to have opened a new era in the treatment of schizophrenia.[1,2] Two groups of neuroleptics are distinguished: (i) classical drugs like haloperidol and chlorpromazine, still widely used for the treatment of schizophrenic patients, and (ii) "atypical neuroleptics".[2] The latter show the same (or superior) therapeutic properties compared to the classical neuroleptics, but do not produce the severe side effects of the latter[3] such as extrapyramidal disorders or prolactine secretion.

However, these atypical new neuroleptics must be administered carefully, because they can also produce other adverse effects[4,5] such as agranulocytosis and convulsions. These damages are found if the daily administration is too high or unsuitable for certain schizophrenic patients. Often other central nervous system (CNS) drugs are administered, which can alter the metabolism of the neuroleptics.

It is evident that for the quality control of these drugs in pharmaceutical formulations adequate analytical methods are necessary, which can distinguish, identify, and quantify individual species in the particular class of drugs.

Several papers report on the analysis of CNS drugs; most of them use HPLC methods for determination of a single compound (or a few compounds) in pharmaceutical dosage forms or synthetic mixtures[6-11] Two papers[12,13] describe HPLC procedures for the separation of various tricyclic drugs and application to commercial preparations.[13] An electrophoretic method is also reported and applied to the separation of ansyolitic drugs.[14]

Capillary electrophoresis, in fact, often offers an alternative to HPLC procedures. It was the aim of this work to develop an electrophoretic method suitable for identification and quantitation of the neuroleptics in order to dispose a reliable tool for the quality control of different pharmaceutical forms.

## EXPERIMENTAL

### Reagents

The following pharmaceutical formulations were examined: Largactil (Rhone-Poulenc Rorer, Milan, Italy) as tablets containing 25 mg of chlorpromazine. Largactil oral solution containing 40 mg/mL of chlorpromazine. Leponex (Novartis Farma, Origgio, Varese, Italy) as tablets containing 100 mg of clozapine. Loxapac (Lederle Laboratories, Gosport, England) as capsules containing 10 mg of loxapine. Risperdal (Janssen-Cilag, Borgo S.Michele, Italy) as tablets containing 1 mg of risperidone. Serenase (Lusofarmaco, Milan,

Italy) as tablets containing 5 mg of haloperidol and as oral solution containing 2 mg/mL of haloperidol. Zyprexa (Eli Lilly, Sesto Fiorentino, Italy) as tablets containing 5 mg of olanzapine.

The following pure substances were used: olanzapine (Eli Lilly, Indianapolis, IN, USA), loxapine (Lederle Laboratories, Pearl River, NY, USA), clozapine (Novartis, Milan, Italy), risperidone (Janssen-Cilag, Sidney, Australia), chlorpromazine and haloperidol (Sigma, St. Louis, MO, USA).

Sodium hydroxide (analytical grade) and ortho-phosphoric acid (85 %), were purchased from E. Merck (Darmstadt, Germany). Water was doubly distilled from a quartz apparatus.

The buffer was prepared by dissolving the suitable volume of o-phosphoric acid in water and adjusting the pH with sodium hydroxide (1 mol/L). CZE buffers and all the solutions obtained from pharmaceutical formulations were filtered (0.45 μm, Minisart RC25, Sartorius, Göttingen, Germany) prior to use.



## Apparatus and Capillary Handling

Capillary zone electrophoresis was carried out with an electrophoretic apparatus (P/ACE System 2100, Beckman Instruments, Fullerton, CA, USA). Separation was performed in a 50 μm I.D. uncoated fused-silica capillary (Composite Metal Services, Hallow, UK), with a total length of 28.3 cm and an effective length of 22 cm. Voltage was +15 kV with currents typically less than 50 μA. For quantitative analysis the sample solutions were injected into the capillary by pressure for 10 sec (3.5 kPa**)**. The detector was operated at 214 nm.

Before use, the capillary was rinsed with deionized water, with 1 mol/L sodium hydroxide, and water before filling in the CZE buffer. After each run the capillary was rinsed with buffer (2 min). For storage overnight, the capillary was washed with water and additionally with sodium hydroxide, and again with water (rinsing time was 10 minutes, each).

## Solutions and Sample Pretreatment

The stock solutions of the neuroleptics were prepared from pure standard compounds by dissolving 10 mg of substance in 10 mL of buffer. The standard solutions were prepared by diluting suitable amounts of stock solutions with deionized water. Stock solutions of neuroleptics obtained from pharmaceutical formulations, and in particular from tablets, were prepared by finely grinding 10 units. An amount of powder equal to 10 mg of declared active principle (or equal to 5 mg of declared active principle for Risperdal) was weighed. The material was transferred into a test tube, 10 mL of BGE (or 5 mL of BGE for Risperdal) were added, and after agitation for 10 minutes on an ultrasonic bath

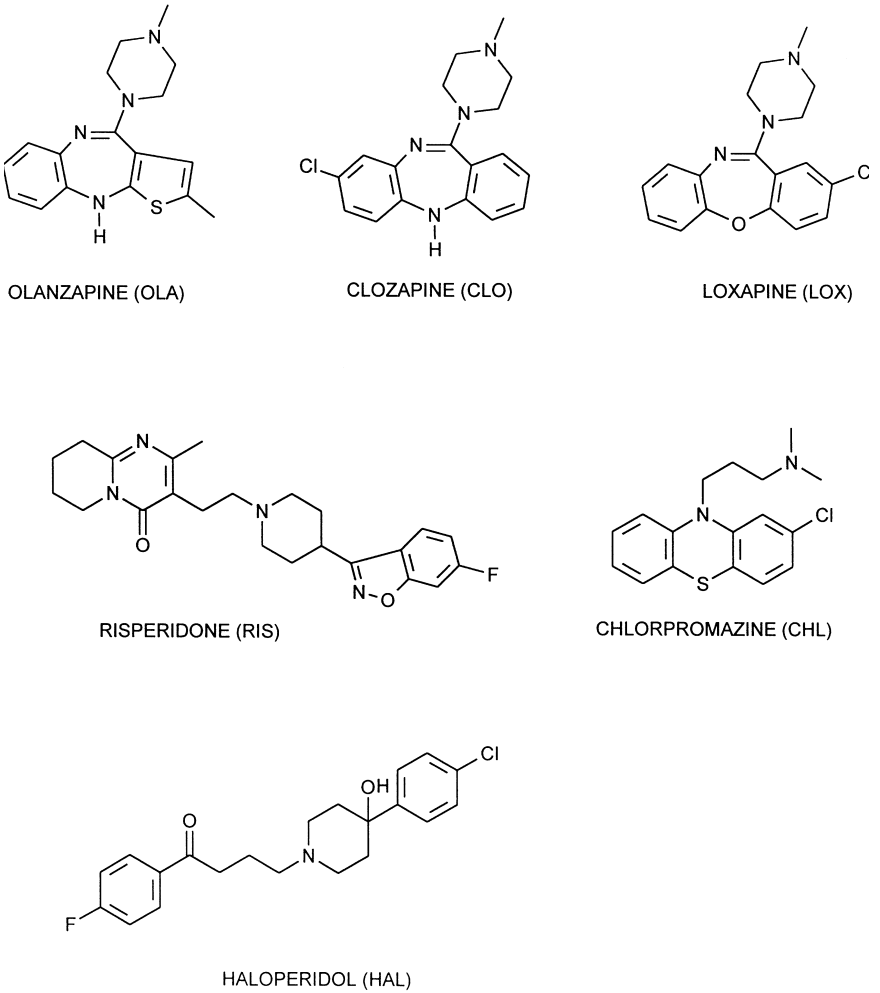


**Figure 1.** Chemical structures of neuroleptics.

the mixture was filtered. In this way a solution of declared concentration of 1 mg/mL was obtained; the working solutions were prepared by dilution with water. The stock solution of the solid pharmaceutical forms available like capsules was prepared mixing finely the content of 10 capsules and using the same procedure described above.

The stock solutions of the liquid pharmaceutical formulations were prepared by taking a volume of the oral solution, equal to 10 mg of declared active principle. This volume was transferred into a test tube with 10 mL of buffer; and after agitation for 15 min with vortex, the solutions were filtered, and the working solutions were prepared by dilution with deionized water.

## RESULTS AND DISCUSSION

### Separation Selectivity

We recently developed an electrophoretic method for the separation of various CNS drugs with neuroleptic and antidepressant activity.[15] It allowed the separation of eleven compounds in an acidic electrophoretic buffer either with ß-cyclodextrin or polyvinylpyrrolidone as additive. These conditions, worked out by variation of a number of experimental variables like pH, ionic strength, additive concentration influencing separation, can be adopted to the present problem of the quantitation of the six neuroleptics (Figure 1) in a number of pharmaceutical formulations like tablets, capsules or oral solutions.

Note that for this purpose it is in principle not necessary to separate all interesting compounds from each other, because these drugs are present in a particular formulation only as single agent, and not together with others. Thus the analytical goal is directed to e.g. short analysis time (including sample preparation) rather than to unnecessarily high resolution.

Note further that the quantitation of the drugs in pharmaceutical formulations is normally not a question of detection sensitivity, because the agents are present in the formulations in relatively high concentration. Under these circumstances a very simple background electrolyte suffices for the separation and determination of all analytes.

It was found by varying the pH and the ionic strength that a phosphate buffer at pH 2.5 and an ionic strength of 35 mmol/L, without a further additive, is selective enough to separate all compounds of interest.

An electropherogram of a mixture of the six neuroleptic drugs obtained under the chosen conditions is shown in Figure 2. It is seen that all compounds are baseline separated within about 3 min.

The relative migration times allow the identification of each drug. These migration times are related either to haloperidol or to risperidone used as an internal standard, depending on the analyte to be determined.

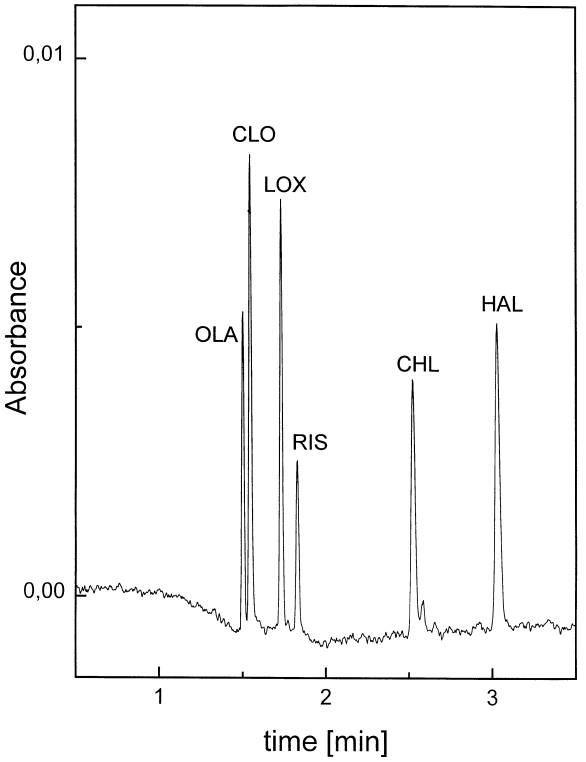


**Figure 2.** Electropherogram of a standard mixture of the six analytes. Conditions: uncoated fused-silica capillary (28.3 cm total length, 22.0 cm effective length, 50 μm I.D.), phosphate buffer (pH 2.5, ionic strength 35 mmol/L). Voltage +15 kV. UV detection at 214 nm. Sample concentration 10 μg/mL each. Symbols of the solutes as in Figure 1.

## Quantitation

Calibration curves were performed using aqueous standard solutions, with at least 6 different concentrations in the range between 5-50 μg/mL. Table 1 summarizes the parameters of the calibration lines obtained by means of least square.

It can be seen that good linearity is found, with correlation coefficients higher than 0.999. The repeatability of the determination of the peak area was derived by analyzing a drug solution of 10 μg/mL concentration for n = 6. It is better than 2% relative standard deviation (RSD).

**Table 1**

**Parameters for Determination of the Neuroleptic Drugs***

| Drug | Rel. Migration Time[a] | Equation | r | RSD % |
|---|---|---|---|---|
| Olanzapine | 0.49[b] | Y = 0.00463 + 0.0151 X | 0.9992 | 1.2 |
| Clozapine | 0.51[b] | Y = 0.00273 + 0.0264 X | 0.9996 | 1.3 |
| Loxapine | 0.57[b] | Y = 0.0022 + 0.0135 X | 0.9999 | 1.2 |
| Risperidone | 0.60[b] | Y = 0.00269 + 0.0108 X | 0.9999 | 2.0 |
| Haloperidol | 1.38[c] | Y = 0.00112 + 0.0160 X | 0.9998 | 1.1 |
| Chlorpromazine | 1.65[c] | Y = 0.00088 + 0.0251 X | 0.9999 | 1.4 |

* Calibration lines of the reference compounds are derived with X = concentration of the sample in µg/mL, and Y = peak area (in aborbance units $min^b$ $10^3$).
[a] Time is given relative to that of [b]haloperidol (as I.S.), [c]risperidone (as I.S.).



**Application to Pharmaceutical Formulations**

The electrophoretic method described above was applied to the determination of the active compounds in eight commercial formulations: six solid forms (tablets A, B, D, E and F; capsules C) and two liquid forms (oral solutions G and H). The method allows applying a very feasible sample pretreatment for all solid formulations, which simply consists of a one-step extraction, filtration, and dilution procedure. This is possible due to the favorable property of capillary electrophoresis under the given conditions. At the low pH of the BGE, no electroosmotic flow occurs, leading to migration into the separation system of exclusively ionic compounds. Therefore non-ionic substances like saccharides, dyes or other filling material, which often represent the major components in pharmaceutical formulations, are not in contact with the separation system.

This favorable situation contrasts e.g. to HPLC, where such compounds are transported by the mobile phase into the column, possibly modifying the stationary phase, and resulting in low reproducibility. Two typical examples are shown in Figure 3.

The data obtained for quantitation of the neuroleptics in formulations are summarized in Table 2. It can be seen that the precision of the total analysis (given by the RSD), including sample pretreatment, is in the same range as that of the electrophoretic determination. The quantities of the drugs found are in accordance with the values claimed by the manufacturers. For each formulation, the limits prescribed by U.S.P. (United States Pharmacopoeia 1995)[16] are fulfilled.

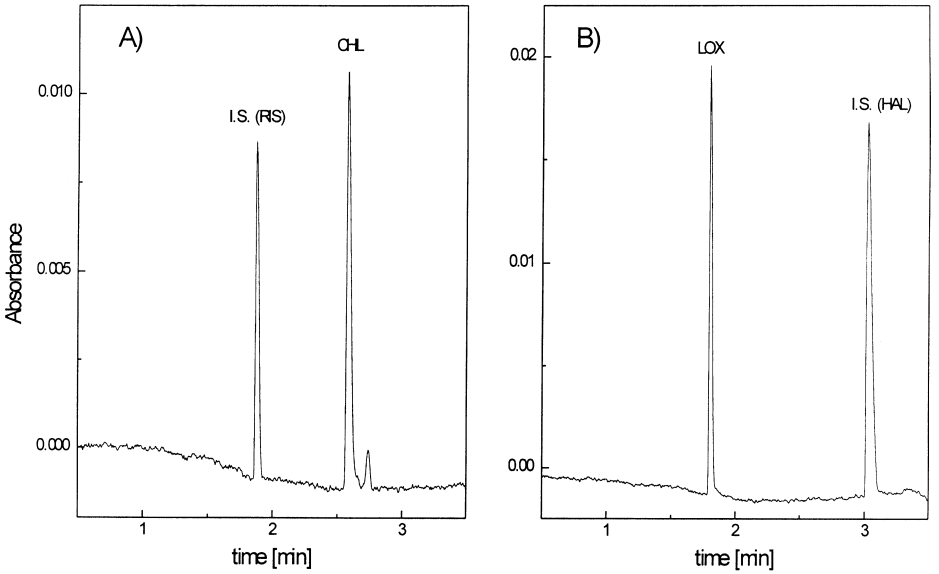


**Figure 3.** Typical electropherograms of the neuroleptic agents in pharmaceutical formulation. A) Largactil oral solution with chlorpromazine (CHL), risperidon was added as I.S.; B) Loxapac capsules with loxapine (LOX) as active agent, haloperidol was added as I.S. Conditions as in Figure 2.



## Table 2

### Quantitation of Neuroleptics in Pharmaceutical Formulations

| Formulation | Active Agent | Mfrs. Label Claim | % Found[a] | RSD % | USP Range % | Recovery[b] | RSD %[c] |
|---|---|---|---|---|---|---|---|
| Tablets A | (OLA) | 5 mg/tablet | 99.8 | 1.6 | 85 - 115 | 98% | 1.2 |
| Tablets B | (CLO) | 100 mg/tablet | 97.0 | 1.7 | 85 - 115 | 102% | 1.5 |
| Capsules C | (LOX) | 10 mg/capsule | 96.7 | 1.3 | 90 - 110 | 104% | 1.4 |
| Tablets D | (RIS) | 1 mg/tablet | 94.0 | 2.1 | 85-115 | 101% | 2.1 |
| Tablets E | (CHL) | 25 mg/tablet | 99.1 | 1.9 | 95 - 105 | 99% | 1.7 |
| Tablets F | (HAL) | 5 mg/tablet | 90.6 | 1.2 | 90-110 | 101% | 1.1 |
| Oral Solution G | (CHL) | 40 mg/mL | 103.0 | 1.7 | 90-110 | 105% | 1.5 |
| Oral Solution H | (HAL) | 0.2% | 105.0 | 1.1 | 90-110 | 101% | 1.1 |

[a] Of declared. Mean of six determinations. [b] For determination of accuracy. [c] Precision of measurements of yield (n=6).

The accuracy of the method was evaluated by means of recovery determinations, adding a known quantity of the reference drug to a certain amount of the pharmaceutical formulation. The recovery values indicate a quantitative yield of the agents indicating a good accuracy of the electrophoretic method. The overall precision (expressed by the RSD values, which were <2% for n=6) is in the same order as the determination of the drug content. It should be mentioned that these results obtained with the present method agree well with those obtained for OLA and CLO using a derivative spectrophotometric method[17] and HPLC procedure[6] respectively. The values reported in the literature are: OLA, 98% recovery, RSD 1.32 %; CLO, 99.3% recovery, 1.33 % RSD.

## CONCLUSION

The proposed method is suitable for a rapid and reliable quantitation of classical and atypical neuroleptic drugs for quality control of commercial formulations. The method consists of a very simple sample preparation, followed by a rapid determination of the analytes within at least 3 min. The reproducibility of the entire quantitation is better than 2% RSD, with a yield of about 100%. Although the sample pretreatment is limited to a simple extraction with buffer, filtration, and dissolution, no interference with other compounds stemming from the sample matrix was observed.



## ACKNOWLEDGMENTS

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