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A rapid LC method for the identification and determination of CNS drugs in pharmaceutical formulations

M.A. Raggi [a,*], G. Casamenti [a], R. Mandrioli, C. Sabbioni [a], V. Volterra [b]

[a] *Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Psychiatric Clinic, University of Bologna, Viale C. Pepoli 5, 40131 Bologna, Italy*

Abstract

Antidepressant, neuroleptic and antiepileptic drugs were identified and determined in pharmaceutical formulations (tablets, capsules and oral solutions) by a rapid high-performance liquid chromatography method. The sample pretreatment consisted of a one-step extraction, filtration and dilution. The chromatographic conditions were: reversed-phase C8 column (150 × 4.6 mm i.d., 5 μm); acetonitrile–tetramethylammonium perchlorate aqueous solution (pH 2.8; 12.6 mM) (45:55, v/v) as the mobile phase; detection wavelength, 230 nm. Calibration curves were linear in the 100–1000 ng ml^{-1} range for all tested drugs except for phenobarbital. The repeatability (or intra-day precision), expressed by the relative standard deviation, was better than 2.0%. The accuracy, resulting from recovery studies, was between 98.1 and 101.3%. The amount of drug found agreed with the declared content within the limits specified by United States Pharmacopeia and British Pharmacopeia. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The past few years have seen great improvements in the pharmacological treatment of neurological and psychiatric disorders. Several new drugs, having different chemical structures and chemical targets, have been introduced in therapy.

The most frequent neurobiologic target for the treatment of depression is the serotonergic system [1], as demonstrated by the recent extended use of selective serotonin re-uptake inhibitors (SSRI) drugs such as fluoxetine. These drugs are administered at lower daily doses and cause less side effects [1,2] than the classical tricyclic antidepressants (e.g. imipramine).

The introduction of new antipsychotic drugs, such as clozapine [1,2] and risperidone [1], also called 'atypical' neuroleptics, has improved the quality of life of many schizophrenic patients. These drugs are particularly important in the treatment of those patients that are non-responders to the treatment with haloperidol or other classical neuroleptic drugs, and/or suffer from severe extrapyramidal effects caused by these drugs [2]. Furthermore, anticonvulsant drugs are sometimes administered as mood stabilizers in the

* Corresponding author. Tel.: +39-051-2099-700; fax: +39-051-2099-734.
E-mail address: raggima@kaiser.alma.unibo.it (M.A. Raggi)

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psychiatric clinics. From this stems the need for simple and reliable analytical methods for rapid and accurate quality control of both new and classical central nervous system (CNS) drugs.

Several papers report on the analysis of CNS drugs; most of them use high-performance liquid chromatography (HPLC) methods for the determination of a single compound (or a few compounds) in pharmaceutical dosage forms or synthetic mixtures [1–12]. Two papers [13,14] describe HPLC procedures for the separation of various tricyclic drugs and their application to commercial preparations [14]. Recently, a flow injection fluorimetric determination of benzodiazepines has been reported [15]. Electrophoretic methods have also been reported for the separation of anxiolytic [16] and neuroleptic [17,18] drugs.

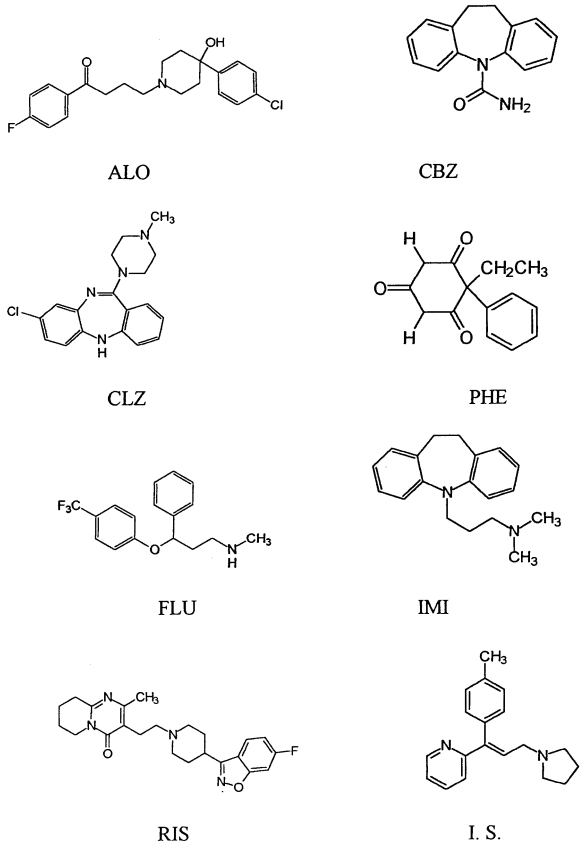

Fig. 1. Structures of the investigated compounds.

The goal of this study is the development of a feasible and reliable HPLC method for the rapid analysis of CNS drugs, with different chemical structures, contained in pharmaceutical formulations, in order to identify and determine the individual drugs with a unique and reliable procedure.

For this purpose, we studied commercial formulations available in Italy, containing atypical neuroleptics (clozapine (CLZ) and risperidone (RIS)), classical neuroleptics (haloperidol (ALO)), tricyclic antidepressants (imipramine (IMI)), SSRI antidepressants (fluoxetine (FLU)) and antiepileptics (phenobarbital (PHE) and carbamazepine (CBZ)). The chemical structures of the examined drugs are reported in Fig. 1.

2. Experimental

2.1. Chemicals

Eli Lilly Italia S.p.A. (Sesto Fiorentino, Florence, Italy) kindly donated fluoxetine hydrochloride. Novartis Italia S.p.A (Origgio–Varese, Italy) kindly donated clozapine. Carbamazepine, triprolidine hydrochloride, haloperidol and imipramine hydrochloride were purchased from Sigma Chemicals. Risperidone and phenobarbital were supplied by Janssen-Cilag Italia (Borgo S. Michele, Latina, Italy) and Rhône-Poulenc Rorer Italia (Milan, Italy), respectively. Carlo Erba (Milan, Italy) supplied methanol, acetonitrile, 25% (m/m) ammonia and 65% (m/m) perchloric acid, all of analytical grade. Tetramethylammonium perchlorate was purchased from Sigma (St. Louis, MO, USA). Ultrapure water was obtained by means of a MilliQ apparatus from Millipore (Milford, MA, USA). Whatman paper filters 41 (55 mm) were used.

The following pharmaceutical formulations were examined: Leponex® as 100 mg tablets of clozapine, Tegretol® as 200 mg tablets of carbamazepine, and Tofranil® as 25 mg tablets of imipramine (Novartis Farma, Origgio, VA, Italy); Belivon® (Janssen-Cilag) as 2 mg tablets of risperidone; Serenase® (Lusofarmaco, Milan, Italy) as oral drops of 2 mg/ml haloperidol; Prozac® as 20 mg capsules of fluoxetine (Eli Lilly S.p.a.); Garde-

nale® as 50 mg tablets of phenobarbital (Rhône-Poulenc Rorer SpA, Origgio, Italy). Triprolidine was used as internal standard.

2.2. Apparatus and chromatographic conditions

The chromatographic system for HPLC analysis was composed of a Jasco (Tokyo, Japan) model PU-980 chromatographic pump and a Jasco UV-975 detector.

Separations were obtained on a Varian (Harbor City, CA, USA) ResElut (C8 150 × 4.6 mm i.d., 5 μm) reversed-phase column. The samples were injected into the column through a 20 μl loop. The mobile phase was prepared as follows: 440 mg of tetramethylammonium perchlorate and 0.2 ml of 7% (m/m) $HClO_4$ were added to about 100 ml of ultrapure water; the resulting solution was brought up to pH 2.8 with 2.5% (m/m) ammonia, then diluted to 200 ml with water. This aqueous solution was mixed with 164 ml acetonitrile, filtered through a Phenomenex membrane filter (47 mm membrane, 0.2 μm, NY) and degassed by an ultrasonic apparatus. The flow rate was 1.3 ml min^{-1} and the detection wavelength was 230 nm.

Data processing was carried out by a Varian Star Chromatography software installed on a 40486 IBM computer. A Crison (Barcelona, Spain) MicropH 2000 pHmeter and an ALC (Milan, Italy) model 4225 centrifuge were used. A Jasco UVIDEC-610 double-beam spectrophotometer was used for preliminary studies, to choose the detector wavelength and for the stability control of the stock solutions.

2.3. Solutions and sample pretreatment

The stock solutions of the drugs were prepared from pure compounds by dissolving 20 mg of substance in 20 ml of methanol. The standard solutions were prepared by diluting suitable amounts of stock solutions with the mobile phase. To each standard solution was added triprolidine as internal standard at a concentration of 250 ng ml^{-1}. The drug solutions obtained from pharmaceutical formulations, and in particular from tablets, were prepared by finely grinding 10 units of the particular drug. An amount of powder equal to 20 mg of declared active principle was weighed. The material was transferred to a test tube, 20 ml of methanol added and, after agitation for 5 min, centrifugation at 3000 × g for 15 min, the mixture was filtered. In this way, a solution of declared concentration of 1 mg ml^{-1} was obtained; the working solutions were prepared by dilution with the mobile phase. The stock solution obtained from the solid pharmaceutical forms available such as capsules (Prozac®) was prepared by finely mixing the content of ten capsules and using the same procedure already described. The stock solutions from the liquid pharmaceutical formulation (Serenase®) were prepared by diluting an aliquot of the oral solution (containing 10 mg of declared active principle) in a test tube with 10 ml of mobile phase. After agitation for 5 min, the solution was further diluted with the mobile phase.

Triprolidine was added to the solutions obtained from the pharmaceutical formulations. The internal standard was present at a concentration of 250 ng ml^{-1}.

3. Results and discussion

3.1. Chromatographic conditions

We have recently studied the separation of various CNS drugs with neuroleptic, antiepileptic and antidepressant activity [19]. This investigation was based on the employment of an HPLC method that allowed for the separation of 12 compounds in human plasma. The leading conditions of this method, which resulted as optimal for toxicological analysis in human plasma, can be used, with some modifications, to quantify the chosen CNS drugs in commercial tablets, capsules or oral solutions. It is important to note that it is not necessary to separate all the compounds from each other, since each formulation contains only one single drug. For this study, a short analysis time (including sample preparation) rather than unnecessary resolution is important. These considerations led to some changes of the chromatographic conditions, namely a notable increase in the organic modifier percentage (from 37 to 45%)

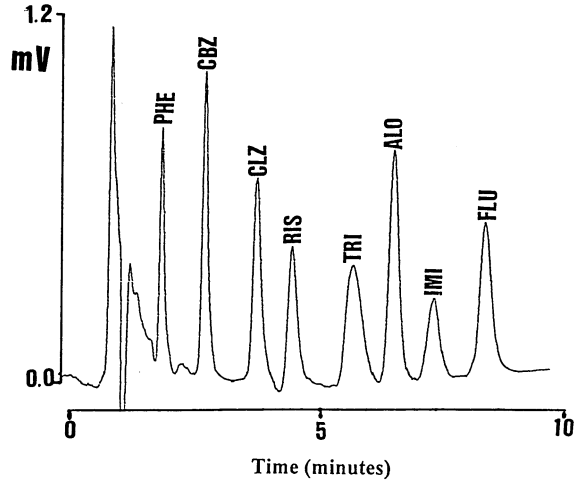

Fig. 2. Chromatogram of a 250 ng ml^{-1} standard solution of the seven compounds examined. Stationary phase: ResElut (150 × 4.6 mm i.d., 5 μm) C8 reversed-phase column. Mobile phase, acetonitrile/12.7 mM, pH 2.8 tetramethylammonium perchlorate (45:55, v/v). Flow rate, 1.3 ml min^{-1}. Detection wavelength, 230 nm. Retention times: PHE, 1.9 min; CBZ, 2.8 min; CLZ, 3.7 min; RIS, 4.5 min; ALO, 6.4 min; IMI, 7.4 min; FLU, 8.3 min; internal standard (TRI), 5.7 min.

in the mobile phase and a higher flow rate (1.3 instead of 1 ml min^{-1}).

The standard working solutions were prepared daily, the stock solutions monthly and the intermediate standard solutions weekly. This working pattern was the result of spectrophotometric stability assays.

A chromatogram of a mixture of the various drugs obtained under the described conditions is presented in Fig. 2, which shows that all compounds are analyzed within about 9 min. The relative retention times allow the identification of each drug. These retention times are related to triprolidine used as the internal standard.

3.2. Calibration curves

Calibration curves were performed for each drug using the standard solutions in the mobile phase, with at least six different concentrations in the range between 100 and 1000 ng ml^{-1}, except for phenobarbital, which needs a range of 250–1250 ng ml^{-1}. Table 1 summarizes the parameters of the calibration lines obtained by means of the least squares, which show good linearity, with correlation coefficients higher than 0.999. The repeatability of the peak area determination was derived by analyzing a CNS drug solution of 500 ng ml^{-1} concentration ($n = 6$). The relative standard deviation (R.S.D.) is better than 2%.

3.3. Application to pharmaceutical formulations

The HPLC method already described was used in the determination of the active principles in seven commercial formulations: six solid forms (five tablets and one capsule) and one liquid form. This method allows analysis using a very feasible sample pretreatment for all solid formulations, which consists simply of a one-step extraction by means of methanol, centrifugation, filtration and

Table 1
Calibration curves of CNS drugs

Drug	Linearity (ng ml^{-1})	Equation $y = a + bx$[a]		r	R.S.D.%
		a	b		
Carbamazepine	100–1000	−83.3	63.3	0.99995	1.1
Clozapine	100–1000	170.0	52.6	0.99995	1.6
Fluoxetine	100–1000	−158.1	29.1	0.99931	1.8
Haloperidol	100–1000	−15.5	15.2	0.99999	1.7
Imipramine	100–1000	−39.0	12.8	0.99911	1.7
Phenobarbital	250–1250	−48.2	12.5	0.99995	1.8
Risperidone	100–1000	−251.4	16.9	0.99910	1.9
Internal Standard	–	–	–	–	1.9

[a] Where y is expressed in arbitrary area units and x is expressed in ng ml^{-1}.

Table 2
Assay characteristics

Formulation	Active agent	Manifacturer's label claim	% found	R.S.D.%	US Pharmacopeia range (%)	British Pharmacopeia range (%)	Accuracy ($n = 6$)	R.S.D.%
Tegretol®	(CBZ)	200 mg/tablet	98.9	1.0	92–108	95–105	102.6	2.0
Leponex®	(CLZ)	100 mg/tablet	98.4	1.3	85–115	–	98.1	0.6
Prozac®	(FLU)	20 mg/tablet	100.9	1.5	90–110	–	99.1	1.9
Serenase®	(ALO)	0.2% w/v	99.4	1.3	90–110	95–105	100.8	2.1
Tofranil®	(IMI)	25 mg/tablet	98.8	1.5	93–107	92.5–107.5	96.1	2.0
Gardenale®	(PHE)	50 mg/tablet	100.0	2.0	90–110	92.5–107.5	97.7	0.9
Belivon®	(RIS)	2 mg/tablet	98.1	1.8	–	–	98.7	1.5

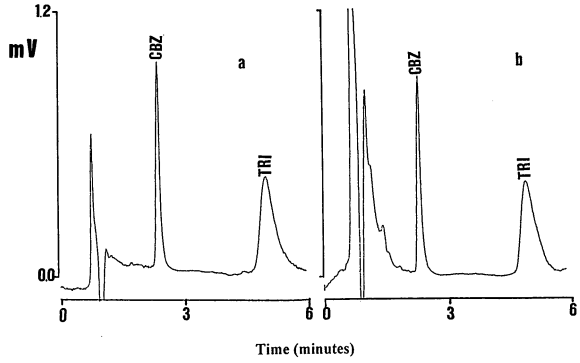

Fig. 3. Chromatogram of a 250 ng ml^{-1} standard solution of carbamazepine (a) and of Tegretol® (b). Stationary phase: ResElut (150 × 4.6 mm i.d., 5 μm) C8 reversed-phase column. Mobile phase, acetonitrile/12.7 mM, pH 2.8 tetramethylammonium perchlorate (45:55, v/v). Flow rate, 1.3 ml min^{-1}. Detection wavelength, 230 nm. Retention times: CBZ, 2.8 min; internal standard (TRI), 5.7 min.

subsequent dilution procedure with the mobile phase. For example, the HPLC analysis of carbamazepine in tablets is reported in Fig. 3(b), while Fig. 3(a) indicates the chromatogram of a carbamazepine standard solution. The peak at retention time of 5.6 min is due to internal standard (triprolidine).

The data obtained for the quantitation of the CNS drugs in formulations are summarized in Table 2. It can be seen that the precision of the total analysis (given by the R.S.D.), including sample pretreatment, is in the same range as the chromatographic determination. The quantities of the drugs found are in accordance with the values claimed by the manufacturers and with the limits prescribed by the United States Pharmacopeia [20] and British Pharmacopeia [21] where available.

The accuracy of the method was evaluated by means of recovery studies, by adding known quantities (100, 250 and 600 ng ml^{-1}) of the standard solutions of the examined drugs to a known amount (250 ng ml^{-1}) of the pharmaceutical formulation. The recovery values obtained on six determinations at every concentration level indicate a quantitative yield of the agents, indicating good accuracy. The overall precision (expressed by the R.S.D.% values, which were $< 2\%$ for $n = 6$) is in the same order as the determination of the drug content. It should be mentioned that the results obtained with the present method agree well with those obtained for neuroleptic drugs using a capillary electrophoretic method [17].

4. Conclusion

The proposed method, in terms of accuracy and precision, is suitable for the quality control of CNS drugs in commercial formulations. In fact, the reproducibility of the analysis is better than 2% R.S.D., with a recovery of about 100%. Furthermore, it has the advantage of being rapid (the HPLC determination lasts less than 9 min; in fact, the compounds were eluted between 1.9 and 8.3 min) and easy, and the sample preparation is limited to a simple extraction and filtration. Although it is very simple, no interference from the excipients of the various examined formulations was observed.

Studies are in progress in order to extend this method to the analysis of other CNS drugs, such as chlorpromazine, olanzapine and paroxetine in pharmaceutical dosage forms. The preliminary results seem to be satisfactory.

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