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Simultaneous LC determination of some antidepressants combined with neuroleptics

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Abstract

A reversed-phase HPLC method with UV detection at 252 nm is presented for the simultaneous determination of some tricyclic antidepressants (amitriptyline, imipramine) and neuroleptics (chlorprothixene, thioridazine) in their quaternary mixtures. Sample analysis was performed on a bonded reversed phase C-18, 5 μm, 250 × 4.6 mm ID (Lichrospher 100RP-18) column using acetonitrile and 0.01 M aqueous solution of triethylamine (1:1) as the mobile phase at 0.9 ml/min. The pH was adjusted to 2.7 with concentrated phosphoric acid. The retention time was for imipramine, amitriptyline, chlorprothixene, and thioridazine 5.8, 6.5, 8.3, 10.8 min, respectively. The linearity was obeyed up to 15 ppm for imipramine and amitriptyline, 12 ppm for chlorprothixene and 10 ppm for thioridazine. The presented method also allows the determination of the mentioned drugs individually in their pharmaceutical preparations.

Keywords: Imipramine; Amitriptyline; Chlorprothixene; Thioridazine; Tricyclic drugs; Reversed phase HPLC; Simultaneous determination; Pharmaceutical analysis

1. Introduction

Imipramine, chlorprothixene, thioridazine and amitriptyline hydrochlorides, the compounds studied in presented work, are used as psychotropic drugs in treatment of the various mental diseases. Due to their chemical structure, every of mentioned above compound shows different pharmaceutical effect. Amitriptyline and imipramine hydrochlorides–dibenzoazepine derivatives represent class of dibenzocycloheptadiene derivatives and dibenzoazepine derivatives, respectively. They have antidepressive properties and are used widely in treatment of depression. They act more effective when they are combined with tricyclic antipsychotic drugs (e.g. phenothiazine or thioxantene derivatives). The chlorprothixene–thioxantene derivative and the thioridazine–phenothiazine derivative exhibit neuroleptic properties. All groups of the studied compounds are chemically active due to their chemical structure: tricyclic aromatic ring with sulphur and nitrogen heteroatoms (phenothiazine derivatives), double bond between aromatic ring and carbon of aliphatic side chain (thioxantenes), methylene or

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methionine groups (dibenzoazepines). The structure of dibenzocycloheptadienes resembles the structure of phenothiazines with methylene group instead of sulphur and with carbon atom substituting nitrogen in tricyclic aromatic frame. The molecular formulaes of tested drugs are given in Fig. 1.

The therapeutic importance of these drugs requires selective, rapid and accessible methods for clinical monitoring as well as quality control of commercial products. Several procedures were proposed for determination of these drugs. UV–Vis spectrophotometry is the most often used technique for this purpose. Some of the photometric methods of analysis are based on the chemical oxidation of studied compounds to the colour (thioridazine, imipramine) or colourless (chlorprothixene) products by oxidising agents [1–6]. Numerous spectrophotometric methods are utilising the complexation reactions [7–10] of studied drugs. Nagaraya et al. [11] have presented a short review of described spectrophotometric methods used for determination of tricyclic drugs. Various other instrumental methods like spectrofluorometry [12–15], chemiluminescent [16], radioimmune [17,18] and electrochemical [19–21] were proposed for routine analysis. Some of authors proposed the titrimetric procedures [22–24] for quantification of studied compounds. The mentioned procedures possess many advantages. They are simple, accurate and inexpensive, but their selectivity is usually poor. Due to the therapeutic interest which these compounds present, research on their chemical properties, determination in pharmaceutical preparations and biological samples is necessary for quality control of preparations as well as for better understanding of their therapeutic and toxicological behaviour. For this purpose the most effective are various HPLC methods [25–32]. The chromatographic methods are characterised by high sensitivity, selectivity and economical consumption of chemicals. The possibilities of quantification of few analytes is significant in a combined therapy, which is administered in some cases of complicated schizophrenic depression [33] or some psychosis [34].

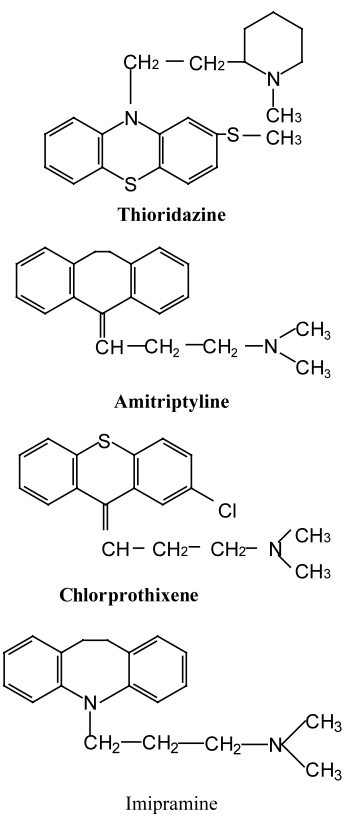

Fig. 1. Chemical formulae of studied drugs.

In this paper, the simultaneous determination of amitriptyline, thioridazine, chlorprothixene and imipramine by a reversed-phase HPLC method with spectrophotometric detection is reported. For this purpose the Sun D. Yoo method [32] of imipramine and desipramine determination was adopted. The validity of this method was proved using synthetic mixtures of the studied drugs and by statistical analysis of the calibration data.

2. Experimental

2.1. Chemicals and reagents

All reagents used were reagent-grade or better. Methanol and acetonitrile were HPLC-grade purchased from Baker (Germany). The water used in all experiments was purified on a Milli-Q system from Millipore. Phosphoric acid (85%) was obtained from POCh (Poland).

Amitriptyline (AMT), imipramine (IMP), chlorprothixene (CPX) and thioridazine (TR) hydrochlorides, triethylamine were obtained from Sigma–Aldrich.

2.2. Standard solutions

Standard solutions (500 ppm) of AMT, IMP, CPX and TR were prepared from the pure product by dissolving appropriate weights in methanol and stored in refrigerator. Working solutions were prepared freshly every day by an appropriate dissolution of standard solution in methanol.

2.3. Chromatography

The chromatographic system, (Thermo Separation) consisted of the 3D detector Spectra System UV 3000, the low-gradient pump P2000, the vacuum membrane degasser SCM Thermo Separation and the Rheodyne loop injector (20 μl), was used. ChromQuest Chromatography Data System software version for Windows NT was used for the acquisition and storage of data. The measurements at 252 nm were carried out using the reversed-phase analytical column, Lichrospher100 RP-18 250 × 4 mm (5 μm) with a guard column 4 × 4 mm (5 μm) (Merck, Germany) and mobile phase included acetonitrile and 0.01 M aqueous solution of triethylamine in proportion 1:1 adjusted to pH 2.7 by drop addition of concentrated phosphoric acid. The flow rate was set at 0.9 ml/min.

2.4. Procedures

2.4.1. Sample preparation: assays in dosage forms

(a) Injection of amitriptyline 'Amitryptylinum®' (Polfa, Poland) (included 50 mg of AMT). An aliquot of one ampoule was quantitative transferred into a 100-ml calibrated flask and diluted with methanol of HPLC-grade to the mark. The working solution was prepared by 50-fold dilution with methanol.
(b) Injection of imipramine 'Imipraminum®' (Polfa, Poland) (included 25 mg of IMP). An aliquot of one ampoule was quantitative transferred into a 50-ml calibrated flask and diluted with methanol of HPLC-grade to the mark. The working solution was prepared by 50-fold dilution with methanol.
(c) Tablets of chlorprothixene 'Chlorprothixen®' (Leciva, Czech Republic) (included 15 mg of CPX). Not less than 20 tablets, each included 15 mg of chlorprothixene, were finely powdered. An accurately weighed portion, equivalent to about 15 mg of chlorprothixene, was transferred into a 100-ml calibrated flask and diluted to the volume with methanol of HPLC-grade. The powder was completely disintegrated by using a mechanical shaker and solution was filtered. The filtrate was transferred into a 100-ml calibrated flask and fulfilled to the mark with methanol. The working solution of 10 ppm was prepared by an appropriate dilution with methanol.
(d) Tablets of Thioridazine 'Melleril®' (Sandoz, France). Not less than 20 tablets, each included 10 mg of thioridazine, were weighed and finely powdered. An accurately weighed portion, equivalent to about 10 mg of thioridazine, was transferred into a 100-ml calibrated flask and diluted to volume with methanol of HPLC-grade. The prepared solution was proceeded according to the procedure described above.

3. Results and discussion

The good chromatographic separation of the mixture depends on mobile phase composition, its pH and its flow rate.

At first, the usefulness for our experiment of some mobile phases described in literature was checked. Among others [26,28–32] the phase proposed by S. D. Yoo et al. [32] for analysis of imipramine and desipramine appeared to be the best. Originally, it consisted of 60% acetonitrile and 40% 0.01 M aqueous solution of triethylamine, with pH adjusted to 3.0 by addition of concentrated phosphoric acid. The use of such composed mobile phase allowed us to obtain the separation of studied drugs. In the next step the

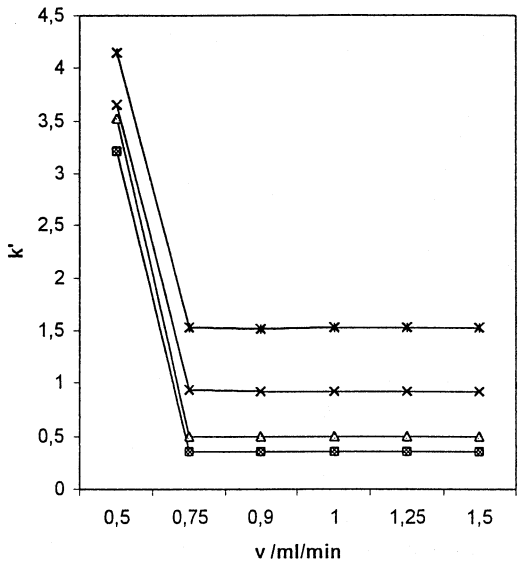

Fig. 2. The influence of flow rate on variation of k' values. □, amitryptyline hydrochloride; △, imipramine hydrochloride; X, chlorprothixene hydrochloride; *, thioridazine hydrochloride.

composition of mobile phase was reoptimised. The optimisation was made using the mixture of studied compounds containing each of drugs at 10 ppm. Conditions for effective separation were determined by examining changes in the content of mobile phase and flow rate on partition coefficient, separation factors and resolution of studied compounds.

It was found that the best separation was achieved for mobile phase consisted of acetonitrile and aqueous solution of triethylamine in proportion of 1:1. The increase in amount of organic solvent resulted in decreasing of peak resolution as well as the increase in volume of amine solution. The time of retention of all compounds raised when acetonitrile content in mobile phase raised. The increase of content of triethylamine in mobile phase gave the same effect: broadening of peaks, extremely long time of analysis (up to 40 min) and increase in partition coefficient (k') value. Similar effect was observed in the case of pH. The varying of pH from 2.7 up to 6 resulted in deteriorating peak shapes and growing of retention times. The pH 2.7 appeared to be optimal for this separation. Therefore, a mobile phase composed of acetontrile–0.01 M aqueous solution of triethylamine in proportion 1:1, with pH 2.7 adjusted by drop addition of 85% phosphoric acid was used for chromatography. The influence of the flow rate was studied in the end of optimisation. It was observed that at flow rate between 0.75 and 1.5 ml/min, there was no considerable variation in the k' values of the compounds analysed. This effect is illustrated in Fig. 2. The flow rate 0.9 ml/min was chosen as optimal. At selected flow rate the retention times of imipramine, amitryptyline, chlorprothixene and thioridazine were 5.8, 6.5, 8.3, 19.8 min, respectively.

The separation for studied set of compounds appeared rather difficult for their similarity of chemical character. The separation of imipramine and amitriptyline was no complete. The resolution factor, R_s, calculated from equation $R_s = 2(t_2 - t_1)/w_1 + w_2$, where t_1, t_2 are the retention times of two neighbouring peaks and w_1, w_2 are the peak widths at the base, for amitriptyline and imipramine was equal 0.8. For others compound the resolution R_s were more then 1.1, signifying complete separation. The chromatographic data of studied compounds separation is presented in Table 1. Despite of incomplete separation of first two compounds, the retention times of the all investigated drugs were found to be reproducible under the presented chromatographic conditions.

Table 1
Chromatographic characteristic of studied compounds separation

Compound	t_R (min)	k'	R_s	a (relative retention)/$a = k'_2/k'_1$ (counted for each pair of peaks)
Imipramine	5.97	0.35	–	–
Amitriptyline	6.62	0.50	0.8	1.4
Chlorprothixene	8.48	0.92	1.5	1.8
Thioridazine	11.1	1.51	1.8	1.6

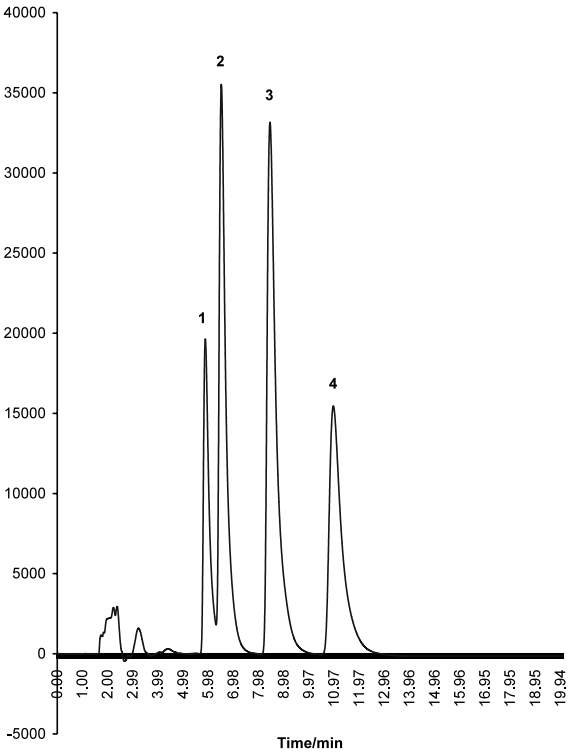

Fig. 3. Typical chromatogram of separated compounds: 1, imipramine; 2, amitryptyline; 3, chlorprothixene; 4, thioridazine (concentration of each compound 10 ppm).

Typical chromatogram of separated compounds is shown in Fig. 3.

Under the optimal chromatographic conditions, the calibration graphs for studied compounds were constructed using peak area versus concentration. Table 2 gives the obtained equations and statistical parameters, such as the limit of detection (S_L), the correlation coefficients and the range of linearity. In order to estimate the precision (RSD) of the method, the replicate samples ($n = 5$) included of each compound at 3 ppm level were measured individually.

3.1. Applications

In order to establish the validity of the proposed method, the compounds studied were determined individually in their pharmaceutical formulation: injections of amitryptyline (Amitryptylinum®) and imipramine (Imipramin®) and tablets of chlorprothixene (Chlorprothixen®) and thioridazine (Melleril®). As can be seen from Table 3, the obtained results are in good agreement with the labelled amount. Interference from the drug excipients was not observed in the chromatograms. Next, an analysis of the content of studied compounds in home made methanolic mixtures of pharmaceuticals was done, to complete the validation of the described method. For this purpose, quaternary mixtures were prepared containing 10 ppm of each compound dissolved in methanol. The obtained results are summarised in Table 4. The error of determination in quaternary mixtures, in all cases does not exceed $\pm 3\%$.

Table 2
Analytical characteristic of elaborated method

	Imipramine hydrochloride	Amitriptyline hydrochloride	Chlorprothixene hydrochloride	Thioridazine hydrochloride
Quantification range (ppm)	0–15	0–15	0.5–12	0.5–10
Equation of calibration curve (x, concentration in ppm)	$26719x+4691$	$61972x+5183$	$80290x+7354$	$55824x$–4669
Correlation coefficient	0.9991	0.9995	0.9990	0.9996
RSD (%) at 3 ppm level	0.34	1.87	2.1	1.2
Limit of detection (μg/ml) (taken as $s_L = a+3s_{x/y}$) [35]	0.332	0.443	0.451	0.439
Limit of quantification (ppm) (taken as $s_L = a+10s_{x/y}$) [35]	1.691	1.500	1.500	1.630

Table 3
Results of determination of studied active compounds in commercial formulations

Determined compound	Pharmaceutical formulation	Labelled amount (mg)	Determined amount (mg) ($n = 7$)	Determined amount by pharmacopoeial method [36] (mg) ($n = 7$)	Error (%) (vs. labelled amount)
Imipramine hydrochloride	Injection 'Imipramin®'	25.00	25.50	25.00	+2.0
Amitriptyline hydrochloride	Injection 'Amitryptylinum®'	50.00	50.65	50.48	+0.3
Chlorprothixene hydrochloride	Tablets 'Chlorprothixen®'	15	14.88	15.10	−1.5
Thioridazine hydrochloride	Tablets 'Thioridazin®'	10	9.87	10.1	−1.3

Table 4
Results of determination in quaternary mixtures of studied compounds

Compound	Taken for analysis (ppm)	Determined concentration (ppm) ($n = 7$)	Error (%) (vs. taken for analysis)
Imipramine hydrochloride	10	10.19	+1.90
Amitriptyline hydrochloride	10	9.84	−1.60
Chlorprothixene hydrochloride	10	10.03	+0.33
Thioridazine hydrochloride	10	10.02	+0.22

3.2. Conclusions

In summary, a sensitive and simple reversed phase HPLC method with UV detection was presented for simultaneous determination of imipramine, amitriptyline, chlorprothixene and thioridazine. The method is suitable for the quantitative analysis of each compound in quaternary mixtures as well as for the individual assay of the studied compounds as single components in their different pharmaceutical formulations.

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