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DYNAMIC CATION-EXCHANGE SYSTEMS FOR THE SEPARATION OF DRUGS DERIVED FROM BUTYROPHENONE AND DIPHENYLPIPERIDINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND APPLIED IN THE DETERMINATION OF HALOPEMIDE IN PLASMA

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SUMMARY

Dynamic (solvent generated) cation-exchange systems for the separation of drugs and main metabolites derived from butyrophenone and diphenylpiperidine (haloperidol, pimozide, halopemide) were investigated.

The effect of organic modifier, detergent, counter-ion concentration and of the pH on the retention has been determined. The results show that variation of these parameters permits adjustment of the retention of these drugs over a wide range.

The dynamic cation-exchange system developed was applied to the determination of halopemide and its main metabolite in plasma. The precision and detection limit of the method and the extraction efficiency were established. The time course of halopemide and plasma levels of patients chronically receiving halopemide are reported.

INTRODUCTION

The necessity of determining drugs and their metabolites in body fluids has been generally accepted for different reasons (for example, pharmacokinetics, bioavailability, biotransformation studies, patient compliance) [1—3]. Among the variety of analytical methods applied for this purpose, high-performance liquid chromatography (HPLC) has proved to be pre-eminently suited for this problem [4—6]. In most instances reversed-phase systems with alkyl-modified silicas were applied. Recently, however, the so-called solvent generated ion-exchange systems were found to be very suitable for the chromatography

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of basic compounds in particular [7—10]. The selectivity and column performance of these dynamic ion-exchange systems is sometimes significantly better than those obtained with the normal reversed-phase systems [9, 10].

In the present study we report the results of an investigation into the use of solvent generated cation-exchange systems with sodium dodecyl sulfate for the analysis of drugs and their metabolites derived from the butyrophenones and from the diphenylbutylpiperidines, of which haloperidol and pimozide are well known prototypes.

The ability of the solvent generated cation-exchange system developed for the analysis of a new psychotropic drug halopemide [N-{2-[4-(5-chloro-2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl]ethyl}-4-fluorobenzamide] (Fig. 1,I) in plasma of volunteers, and of patients chronically receiving this new drug, is demonstrated.

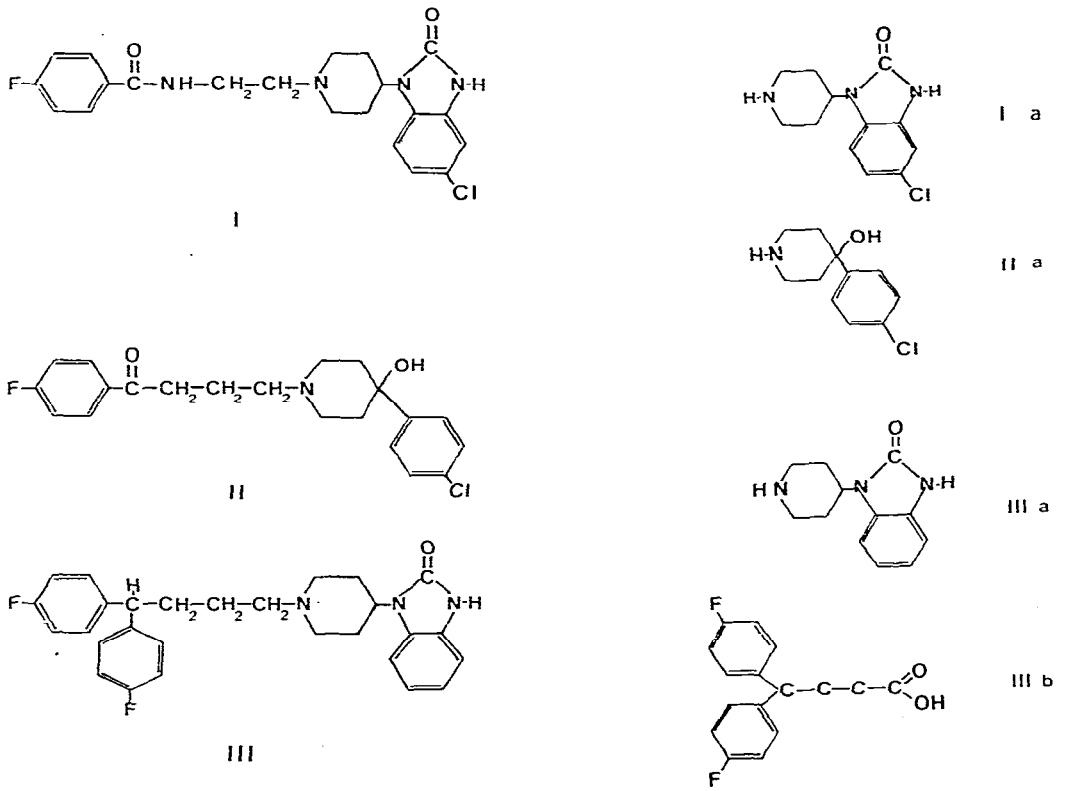


Fig. 1. Structural formulae of the selected compounds. I, Halopemide; Ia, main metabolite of halopemide; II, haloperidol; IIa main metabolite of haloperidol; III, pimozide; IIIa and IIIb, main metabolites of pimozide.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of a reciprocating pump (Orlita, Type AE-10-4.4), a Bourdon type manometer, a high-pressure injection valve

(Rheodyne 7105) equipped with a sample loop of 150 μl, an UV spectrophotometer (Perkin Elmer LC-55), a linear potentiometric recorder (Goerz, Servogor RE542) and an electronic integrator (Autolab I). In all of the experiments stainless-steel columns of I.D. 3 mm and length 125 or 150 mm were used. The wavelength of the UV detector was set at 210 nm.

Materials

In all experiments double-distilled water was used. All solvents and chemicals were of analytical grade and used without any further pre-treatment. Sodium dodecyl sulfate (SDS) was obtained from Merck (Darmstadt, G.F.R.). The octyl-modified silica used as column support was RP-8, mean particle size 5 μm (Merck). The drugs and their metabolites were obtained from Janssen Pharmaceutica (Beerse, Belgium). Their structures are given in Fig. 1.

Procedures

Chromatography. The HPLC columns were packed by a pressurized balanced slurry technique [11] (the slurry liquid consisted of a mixture of chloroform and tetrabromoethane of specific gravity of 1.82); they were washed with 100 ml of methanol and then equilibrated with the eluent until constant retention of the compounds under investigation was obtained. Standard solutions of the drug and its metabolites were prepared from stock solutions of the compounds in methanol (5.7 mg/100 ml) and were stored in a refrigerator at 4°.

Sample preparation. Blank pooled plasma and plasma samples containing halopemide were stored at −20°. After thawing, all samples were ultrasonicated for 3 min. To extract halopemide and its metabolite 0.2 ml of 2 *M* NaOH and 10.0 ml of $CHCl_3$ were added to 1 ml of plasma in a glass stoppered centrifuge tube. The tube was shaken for 1 min and centrifuged (5 min at 1730 *g*). The aqueous phase was removed by aspiration and 9 ml of the organic layer were transferred, using a Hamilton syringe (type 1005), to another tube containing 1 ml of 2.1 *M* perchloric acid. The tube was shaken and centrifuged (for 1 min at 1730 *g*). A 500-μl portion of the aqueous phase was transferred to another tube which contained 250 μl of 4 *M* NaOH and 250 μl of this final solution were used to fill the sample loop.

RESULTS AND DISCUSSION

Chromatography

Dynamic (solvent generated) cation-exchange chromatography with sodium dodecyl sulfate (SDS) was chosen as the separation method for the selected drugs and their respective metabolites (Fig. 1). These dynamic cation-exchange systems were found to be very useful for the separation of amino acids [7] and basic substances such as tricyclic antidepressants [9] and catecholamines [10]. Compared to normal reversed-phase systems, dynamic cation-exchange systems exhibit more flexibility for adjusting selectivity and show significantly better column efficiency (for example, symmetrical peak shapes) [9, 10].

In order to find optimal chromatographic conditions for the separation of the selected drugs and possible metabolites, the influence of a number of param-

eters such as the methanol, SDS and counter-ion concentrations and the pH of the mobile phase on the capacity ratio was investigated.

Fig. 2 shows the influence of the methanol content of the mobile phase. The capacity ratio of basic solutes decreases with increasing methanol content, as is commonly found in reversed-phase systems. However, the decrease of the capacity ratio is mainly due to a decrease of the amount of SDS adsorbed at the hydrophobic support (comparable with the ion-exchange capacity) with increasing methanol content [8].

The influence of the SDS content of the mobile phase is shown in Fig. 3. The capacity ratio of the basic compounds increases with increasing SDS content of the mobile phase and levels off at larger amounts because of the shape of the adsorption isotherm of SDS [8]. For compound IIIb only physical adsorption occurs. Dynamic cation-exchange systems behave in a way similar to conventional ion-exchange systems [7, 8, 10]. This means that the capacity ratio can be adjusted in a predictable way by varying the counter-ion concentration (in this study Na^+) as shown in Fig. 4.

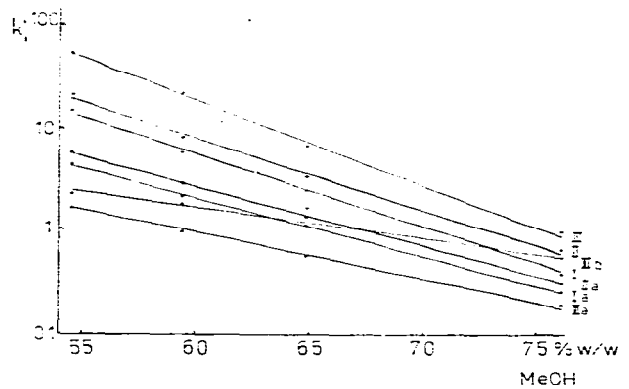


Fig. 2. Influence of the methanol concentration on the capacity ratio (k'_i). Stationary phase: C_8-modified silica. Mobile phase: 0.005 *M* NaH_2PO_4 + 0.1% (w/v) SDS + 0.2 *M* $NaClO_4$ (pH 6.5). Roman numerals as in Fig. 1.

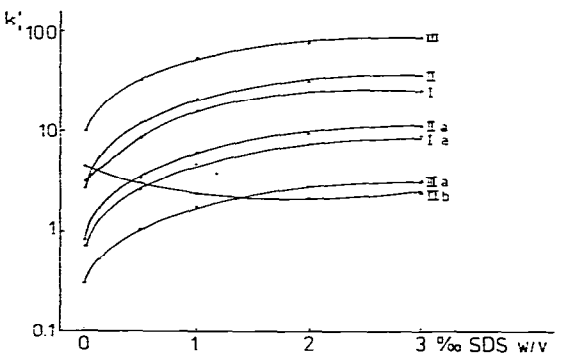


Fig. 3. Influence of the SDS concentration on the capacity ratio. Stationary phase: C_8-modified silica. Mobile phase: 0.005 *M* NaH_2PO_4 + 0.1 *M* $NaClO_4$ + 64.8% (w/w) methanol (pH 6.5). Roman numerals as in Fig. 1.

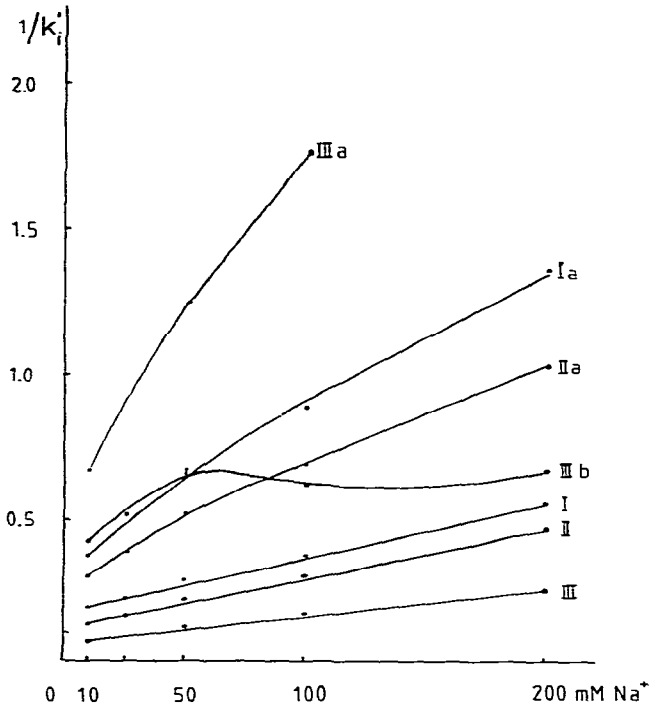


Fig. 4. Influence of the sodium ($NaClO_4$) concentration on the capacity ratio. Stationary phase: C_8-modified silica. Mobile phase: 0.005 *M* NaH_2PO_4 + 0.1% (w/v) SDS + 64.8% (w/w) methanol (pH 6.5). Roman numerals as in Fig. 1.

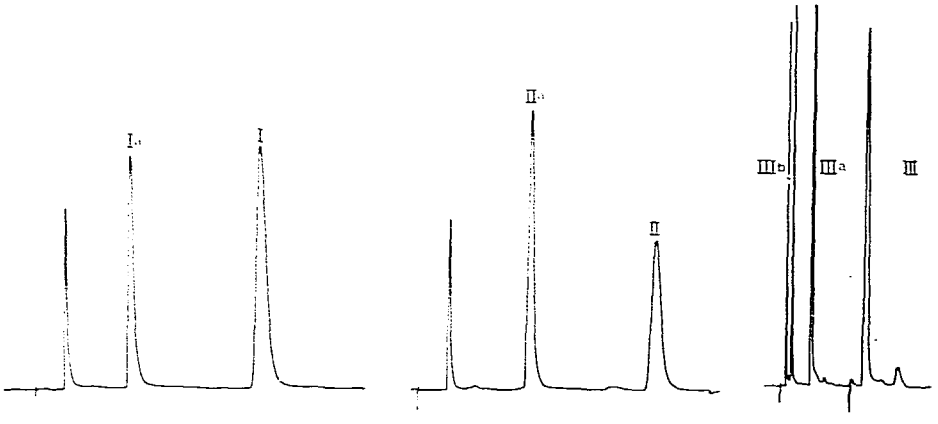


Fig. 5. Separation of test mixtures of selected compounds. Roman numerals are explained in Fig. 1. Stationary phase: C_8-modified silica. Mobile phase: 0.005 *M* NaH_2PO_4 + 0.3% (w/v) SDS + 2 *M* $NaClO_4$ + 59.4% (w/w) methanol (pH 3.00). Flow-rate, 0.60 ml/min.

The influence of the pH of the mobile phase on the capacity ratio was investigated using phosphate-buffered mobile phases of different pH values (range 2.0—6.5). No significant change in capacity ratio could be observed. At pH > 6.5 a significant decrease of the column stability was found. In agreement with previous observations [10] we found a better peak shape and column stability when relatively high sodium perchlorate concentrations were used in the mobile phase.

The results of the influence of methanol, SDS and Na^+ concentration on the capacity ratio as reflected in Figs. 2—4 demonstrate the great flexibility of dynamic cation-exchange systems for the separation of the selected drugs and metabolites. Optimal separation conditions for each of the drugs and its main metabolites can easily be derived from these figures as is demonstrated in Fig. 5.

DETERMINATION OF HALOPEMIDE IN PLASMA

The developed phase system was applied to the analysis of a new psychotropic agent called halopemide, a chemical congener of the neuroleptic benperidol, but with a totally different pharmaceutical and clinical profile [12]. According to the manufacturer this new drug lacks extrapyrimidal side-effects and looks promising as an effective drug for the treatment of psychosis, characterized by autism, emotional withdrawal or apathy.

The metabolism of halopemide has been studied in rats [13]. This study indicates that halopemide is most probably metabolized via an oxidative N-desalkylation into *p*-fluorohippuric acid and compound Ia.

Until now no data about the plasma time course of halopemide in man after a single dose and of plasma levels of patients chronically administered halopemide are known.

The suitability of the dynamic cation-exchange system and of the extraction procedure for the analysis of halopemide in plasma is demonstrated in Fig. 6, which shows chromatograms of extracts of blank and spiked (112 ng halopemide per ml) plasma.

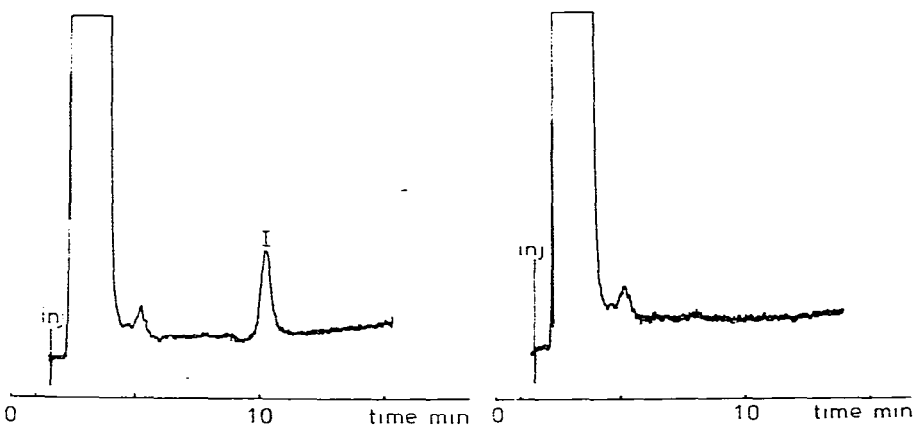


Fig. 6. Chromatogram obtained of an extract of a pooled blank plasma sample and of a plasma sample spiked with 112 ng of halopemide (I). For conditions see Fig. 5.

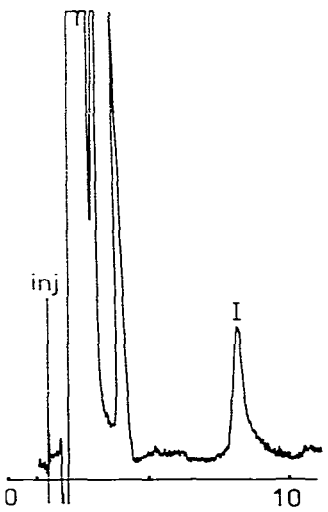
Quantitative aspects

The precision and linearity of the method were determined by injecting 150 μl of solutions of compounds I and Ia at different concentrations and measuring peak height or peak area. The regression of peak area or peak height vs. injected amount was found to be linear for halopemide up to 540 ng/ml, and up to 400 ng/ml for compound Ia, with a correlation coefficient for both compounds of 0.9988, indicating a high degree of linearity. The precision of the method was estimated from repeated injections (n = 10) of solutions of the compounds at high and low concentration levels. For both compounds the standard deviation, using peak height measurements, was 1.7% when 108 ng were injected and 3.0% with 5.0 ng injected. The peak-to-peak value of the baseline noise was 15×10^{-4} a.u. This led to a calculated limit of detection, for a signal-to-noise ratio of 3, of 1 ng for halopemide and 0.75 ng for compound Ia. This corresponds to a detection limit of 7.4 ng/ml plasma for halopemide and 12.9 ng/ml plasma for compound Ia for a given injection volume of 150 μl.

Recovery of the extraction

The recovery and reproducibility of the extraction were determined by spiking blank plasma samples with different amounts of halopemide and compound Ia and extracting as described under procedures. The recovery of halopemide was 98 ± 3% (n = 4) for halopemide and 43 ± 6% (n = 4) for Ia.

The recovery of compound Ia could be increased to 80% by using a more polar extraction liquid composed of diethyl ether and ethyl acetate (10:2, v/v). However, the greater recovery of compound Ia was accompanied by a much



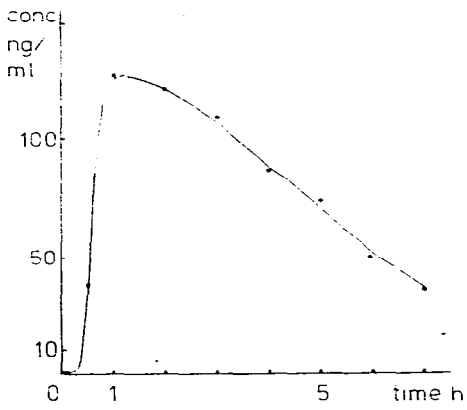


Fig. 7. Chromatogram of an extract of plasma of a patient who chronically received 20 mg of halopemide daily. Comedication was promethazine. For conditions see Fig. 5. Flow-rate 0.48 ml/min.

Fig. 8. Time course of halopemide in plasma of a volunteer after ingestion of a 20-mg dose.

larger background which seriously affects the quantitative determination of both substances. For this reason a milder extraction liquid, despite the low recovery of Ia, was found to be advantageous.

Time course and plasma level of halopemide in volunteers and patients

The developed method for the analysis of halopemide was applied to plasma samples of volunteers and patients. A typical chromatogram of an extract of plasma of a patient is given in Fig. 7. Fig. 8 shows the plasma time course of halopemide in a volunteer after an oral dose of 20 mg. From the data obtained a plasma half-life of 3.5 h could be calculated. The apparent volume of distribution in this case was calculated to be 145 l.

Plasma samples obtained from patients who were chronically treated with halopemide were also determined by HPLC. Promethazine, used as comedication, did not interfere in the analysis. The results of these analyses are given in Table I. In order to determine the main metabolite Ia, a phase system with a 5% lower methanol content was chosen, which guarantees the elution of this compound free from coextracted endogenous plasma constituents.

TABLE I

PLASMA LEVELS DETERMINED BY HPLC OF MALE PATIENTS CHRONICALLY TREATED WITH HALOPEMIDE

Patient No.	Daily dose of halopemide (mg)	Plasma concentration (ng/ml)
1	20	87
2	60	360
3	60	243
4	20	118
5	20	99

In none of the plasma samples of these patients was compound Ia present at levels greater than 12.9 ng/ml (detection limit). This result might indicate a fast elimination rate of this metabolite and/or a slow metabolism of the parent drug.

Analysis of halopemide and this metabolite in urine might give more information about the fate of halopemide in man.

CONCLUSION

Dynamic cation-exchange chromatography was found to be eminently useful for the analysis of the butyrophenon and diphenylalkyl piperidine type psychotropic drugs and their metabolites in plasma.

Plasma levels of halopemide in patients receiving halopemide (20—60 mg daily) chronically, ranged from 87 to 360 ng/ml. Until now no metabolites were found in the plasma at concentrations greater than 12 ng/ml. Future work will therefore be devoted to the analysis of halopemide and its metabolite (Ia) in urine.

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