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Short communication

# Simultaneous determination of haloperidol and bromperidol and their reduced metabolites by liquid–liquid extraction and automated column-switching high-performance liquid chromatography

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**Abstract**

This study describes a new simultaneous determination of haloperidol and bromperidol and their reduced metabolites by modification of automated column-switching high-performance liquid chromatography. The test compounds were extracted from 1 ml of plasma using chloroform–hexane (30:70 (v/v)), and the extract was injected into a hydrophilic metaacrylate polymer column for clean-up and a $C_{18}$ analytical column for separation. The mobile phases consisted of phosphate buffer (0.02 M, pH 4.6), perchloric acid (60%) and acetonitrile (54:1:45 (v/v)) and was delivered at a flow-rate of 0.6 ml/min. The peak was detected using a UV detector set at 215 nm. The method was validated for the concentration range 1–100 ng/ml, and good linearity ($r > 0.999$) was confirmed. Intra-day coefficient variations (CVs) for haloperidol, reduced haloperidol, bromperidol and reduced bromperidol were less than 2.5, 3.1, 2.4 and 2.5%, respectively. Inter-day CVs for corresponding compounds were 3.9, 5.1, 2.6 and 4.4%, respectively. Relative errors ranged from −5 to 10% and mean recoveries were 96–100%. The limit of quantification was 1.0 ng/m for each compound. This method shows good specificity with respect to commonly prescribed psychotropic drugs, and it could be successfully applied for pharmacokinetic studies and therapeutic drug monitoring, particularly in patients receiving both haloperidol and bromperidol.


*Keywords:* Haloperidol; Bromperidol

## 1. Introduction

Haloperidol is still one of the most widely used antipsychotic drugs in the treatment of schizophrenic and other psychiatric disorders [1]. Although reduced haloperidol has little effect as a dopamine D2 antagonist [2], an animal study suggests that reduced haloperidol acts as a potent inhibitor of dopamine uptake [3]. In addition, significant association has been demonstrated between the plasma concentration of reduced haloperidol and extrapyramidal side effects [4]. Bromperidol is a close structure analogue to haloperidol. Based on our previous data showing a strong correlation between plasma concentration of reduced bromperidol and prolactin response [5], the presence of this reduced metabolite suggests some pharmacological activity on dopamine D2 receptor. Thus, it is clinically important to determine plasma concentrations of haloperidol, bromperidol and their reduced metabolites.

Sometimes haloperidol and bromperidol are administered concomitantly when the efficacy of monotherapy is not enough to ameliorate psychotic symptoms. There are numerous references for quantification of either haloperidol or bromperidol by GC, HPLC or LC/MS/MS [6–14]. Among them, a successful separation of its reduced metabolite from haloperidol [9,10,12] or bromperidol [8] and a successful separation of bromperidol from haloperidol [9–11,13,14] have been reported. To date, however, there is no published method for simultaneously analyzing four compounds, haloperidol and bromperidol and their reduced metabolite in biofluids. Therefore, by improving upon our previous methods, this study was designed to achieve simultaneous determination of haloperidol and bromperidol and their reduced metabolites.

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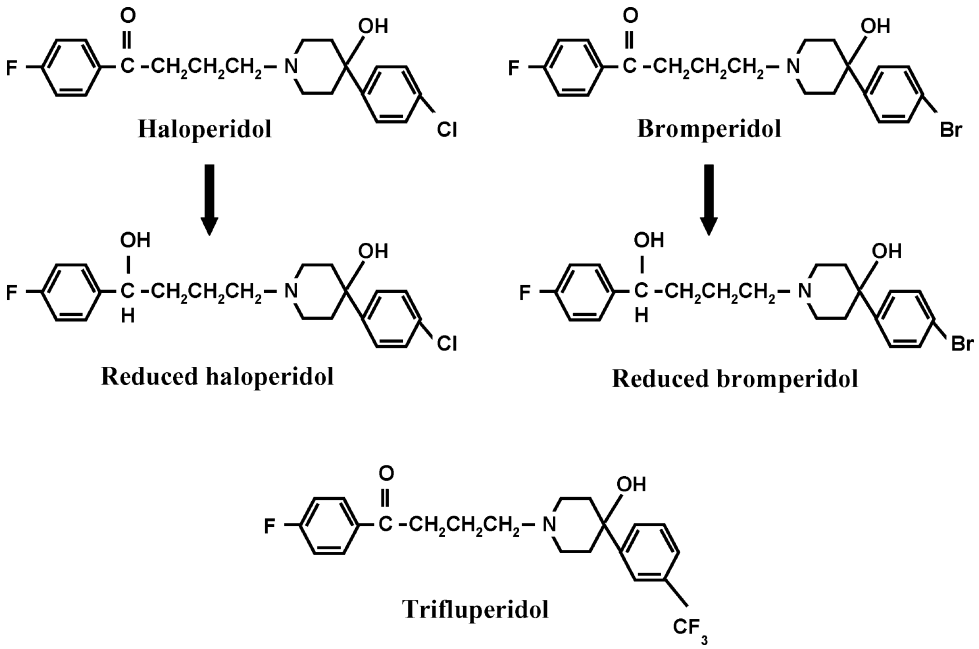


Fig. 1. Chemical structure analogues of haloperidol and bromperidol and reduced metabolites and trifluperidol.

## 2. Experimental

### 2.1. Chemicals

Haloperidol, bromperidol and their reduced metabolites and trifluperidol, 4′-fluoro-4-(4-hydroxy-4-(α,α,α-trifluoro-*m*-tolyl)piperidino)butyrophenone as internal standard (IS) (Fig. 1) were kindly provided by Yoshitomi Pharmaceutical (Osaka, Japan). The purity of these materials was more than 99.5%. Potassium phosphate monobasic, acetonitrile, perchloric acid, *n*-hexane, and chloroform were purchased from Wako Pure Chemical Industries. (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

### 2.2. Drug solutions

Stock solutions of haloperidol, bromperidol and their reduced metabolites and IS for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 0.2 mg/ml. High-concentration working standard solutions of haloperidol, bromperidol and their reduced metabolites and IS (2.0 μg/ml) were obtained by 100 times dilution of each stock solution with purified water. Low-concentration working standard solution (200 ng/ml) of each compound was obtained by further diluting the working standard solution 10 times with purified water. Stock solutions were stable at 4 °C for at least three months. Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 5, 10 and 25 μl of low-concentration working standard solutions (200 ng/ml), and 5, 10, 25 and 50 μl of high-concentration working standard solutions (2.0 μg/ml) in 1 ml of blank plasma (final volume) to yield the final concentrations 1, 2, 5, 10, 20, 50 and 100 ng/ml for each analysis. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-height ratio versus the respective concentration of haloperidol, bromperidol and their reduced metabolites. Stock solution of each compound was separately prepared for quality controls in the same manner as for standard curves. Working plasma solutions were obtained by dilution of stock solutions 1000 times with blank plasma (200 ng/ml). Quality control samples were obtained by spiking 5–100 μl of working plasma solutions in 1 ml of blank plasma (final volume) to yield the final concentrations range of 1, 10 and 20 ng/ml, and kept at −20 °C until analysis. All standard curves were checked using these quality control samples.

### 2.3. Sample collections

The subjects were 18 patients (6 males and 12 females) receiving both oral doses of haloperidol and bromperidol, and all were diagnosed as schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders, Version IV, criteria [15]. The mean (and range) of age and body weight were 49 years (28–66 years) and 60 kg (48–85 kg), respectively. This study was approved by the Ethics Committee of Hirosaki University School of Medicine and written informed consents were obtained from all patients. The mean (and range) of haloperidol and bromperidol were 6.6 (3–15) mg per day and 7.4 (5–12) mg per day, respectively. Concomitant medications were antipsychotics, such as levomepromazine in 4, zotepine in 5, risperidone in 1, sulpiride in 2 and antiparkinson agents, biperiden in 7 and benzodi-

azepines, such as flunitrazepam in 7, nitrazepam in 1, brotizolam in 2, diazepam in 2, alprazolam in 1, and mood stabilizers, such as carbamazepine in 3 and lithium in 1 subjects. Blood samplings (6 ml) were performed 12–14 h after last night dosing of haloperidol or bromperidol. The plasma samples were frozen and kept at −20 °C until analysis.

### 2.4. Apparatus

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluent A and B), a Shimadzu CTO-10A column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan), a Tosoh multiple autovalve PT-8000, and a Tosoh autosampler AS-8020 (500 μl injection volume) (Tokyo, Japan). A TSK gel PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I; 35 × 4.6 mm i.d., particle size 10 μm; Tosoh, Tokyo, Japan) and a $C_{18}$ STR ODS-II column as an analytical column (column II; 150 mm × 4.6 mm i.d., particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan) were used.

### 2.5. Extraction procedure

IS (trifluperidol) 100 μl of 250 ng/ml and 0.5 ml NaOH (2.5 M) were added to 1 ml of plasma and 0.5 ml of water. The tubes were vortex-mixed for 10 s and 5 ml of *n*-hexane–chloroform (70:30 (v/v)) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 1700 × *g* for 10 min at 4 °C, and the organic phase was evaporated in vacuo at 45 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved with 750 μl of eluent A and used as an extract.

### 2.6. Chromatographic condition

Column-switching chromatographic condition was set based on our previous report [8]. A 0.5 ml portion of the extract was automatically injected into the HPLC system. The column-switching system was operated according to the program time depicted in Table 1. From 0 to 10.5 min after the sample injection, haloperidol, bromperidol and their reduced metabolites and IS were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.02 M, pH = 4.6), perchloric acid (60%) and acetonitrile (89:1:10, v/v). Between 10.5 and 18.0 min after the injection, four analytes and IS retained on column I were eluted with a mobile phase (eluent B) of phosphate buffer (0.02 M, pH = 4.6), perchloric acid (60%) and acetonitrile (54:1:45 (v/v)), and effluent from column I was switched to column II. Then haloperidol, bromperidol and their reduced metabolites were separated on column II by eluting with eluent B (between 18.0 and 35.0 min). The flow-rates of eluents A and B were 1.2 and 0.6 ml/min, respectively. The temperatures of column I and II were about 25 °C (room temparature) and 30 °C, respectively. The peak was detected using a UV detector set at 215 nm. The peak height was used for the quantification of these four compounds.

Table 1
Extraction recovery of the four analyte from plasma

| Analyte | Concentration added (ng/ml) | Recovery (%) ($n = 6$) | CV (%) ($n = 6$) |
|---|---|---|---|
| Haloperidol | 1 | 97.9 | 1.7 |
| | 10 | 98.6 | 0.6 |
| | 20 | 99.9 | 1.0 |
| Reduced haloperidol | 1 | 96.2 | 1.9 |
| | 10 | 96.2 | 1.2 |
| | 20 | 96.9 | 1.8 |
| Bromperidol | 1 | 97.6 | 0.9 |
| | 10 | 98.3 | 1.2 |
| | 20 | 97.9 | 1.0 |
| Reduced bromperidol | 1 | 96.7 | 1.9 |
| | 10 | 97.2 | 1.9 |
| | 20 | 97.2 | 1.4 |

### 2.7. Other assay for plasma drug concentrations

Commercial EIA kits (MARKIT®-M Haloperiol II and MARKIT®-M Bromperidol II, Dainippon Pharmaceuticals, Osaka, Japan) were used for determination of plasma concentrations of haloperidol and bromperidol. Limit of detection for each compound was 1.25 ng/ml and CVs was <10%.

## 3. Results and discussion

### 3.1. Chromatography

The chromatogram of an extracted blank plasma sample is shown in Fig. 2A. A representative chromatogram of an extracted blank plasma sample spiked with working aqueous solution containing haloperidol, bromperidol and their metabolites and trifluperidol (internal standard) is shown in Fig. 2B (1 ng/ml) and Fig. 2C (20 ng/ml). The chromatograms of extracted plasma samples obtained from a patient receiving both haloperidol and bromperidol did not show interference peaks (Fig. 2D). All compounds were well separated from each other and from the front of the solvent peaks. Plasma concentrations were 19.6 ng/ml for haloperidol, 8.4 ng/ml for bromperidol, 4.3 ng/ml for reduced haloperidol and 3.8 ng/ml for reduced bromperidol, respectively.

Fig. 3 shows effects of constitution ratio of perchloric acid (0, 0.1, 0.25 and 1.0%) in mobile phase on peak separation of haloperidol and bromperidol, reduced haloperidol and reduced bromperidol after injection of unextracted working aqueous solution containing 100 ng/ml of each compound. A mobile phase having the same pH was adjusted with phos-

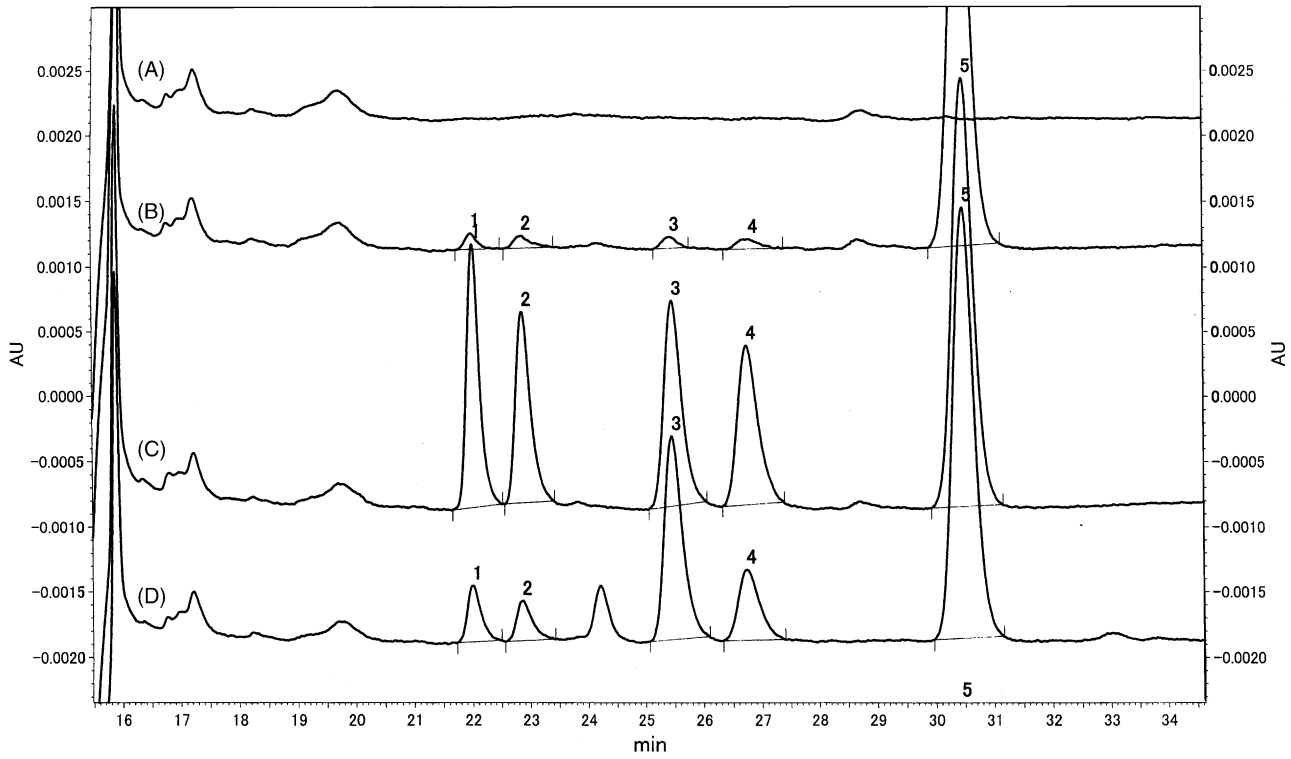


Fig. 2. Representative chromatogram of extracts of blank plasma (A) and extracts of plasma spiked with 1 ng/ml each analyte (B) and 20 ng/ml each analyte (C) and plasma from a patient (D). The peak 1 corresponds with reduced haloperidol; peak 2 with reduced bromperidol; peak 3 with haloperidol; peak 4 with bromperidol; peak 5 with trifluperidol.

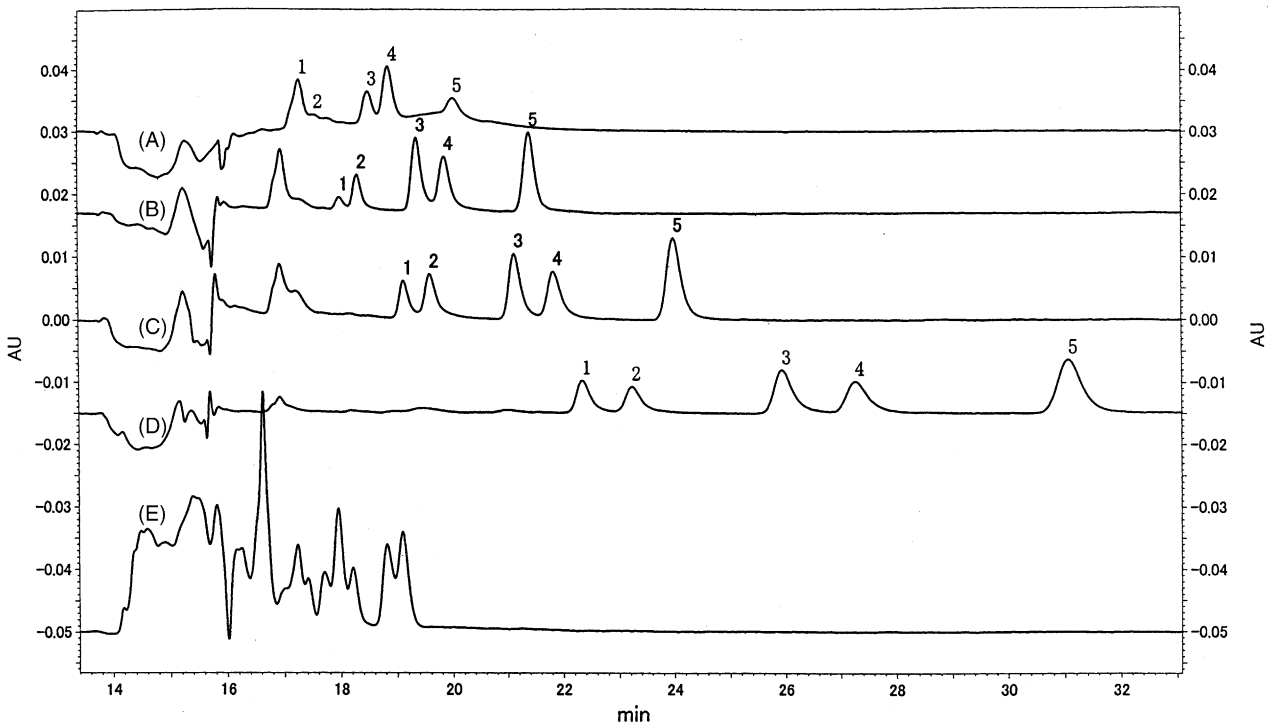


Fig. 3. Effects of composition ratio of perchloric acid (0% (A), 0.1% (B), 0.25% (C) and 1.0% (D)), and phosphoric acid adjusted to the same pH as was used with 1.0% perchloric acid (E) in mobile phase, and effects of a mobile phase within which the pH was adjusted with phosphoric acid to be the same as with 1.0% perchloric acid, on separation of test compounds. The peak 1 corresponds with reduced haloperidol; peak 2 with reduced bromperidol; peak 3 with haloperidol; peak 4 with bromperidol; peak 5 with trifluperidol.

Table 2
Mean values for slope, intercepts and correlation coefficints of six calibration curves for analytes

| Analyte | Slope | Intercept | r |
|---|---|---|---|
| Haloperidol | | | |
| Mean | 0.0119 | −0.0016 | 0.9999 |
| S.D. | 0.0002 | 0.0013 | |
| Bromperidol | | | |
| Mean | 0.0103 | 0.0006 | 0.9996 |
| S.D. | 0.0008 | 0.0048 | |
| Reduced haloperidol | | | |
| Mean | 0.0139 | −0.0080 | 0.9997 |
| S.D. | 0.0004 | 0.0030 | |
| Reduced bromperidol | | | |
| Mean | 0.0088 | 0.0022 | 0.9998 |
| S.D. | 0.0001 | 0.0040 | |

phoric acid, because adjustment with 1.0% perchloric acid did not lead to separation of any these compounds (Fig. 3), suggesting specific effect of perchloric acid but not pH in this method.

### 3.2. Recovery and linearity

Absolute recovery from plasma was calculated by comparing the peak heights of pure standards prepared in purified water, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound ($n = 6$ each). Recoveries and their CV values were determined at three different concentrations ranging from 1 to 20 ng/ml (Table 2). Calibration curves were linear over the concentrations range from 1 to 100 ng/ml ($r = 0.999$ for all compounds) (Table 3).

Table 3
Precision (CV) and accuracy (relative error) for determination of four analytes in spiked plasma ($n = 6$)

| Analyte | Concentration added (ng/ml) | Intra-assay | | Inter-assay | |
|---|---|---|---|---|---|
| | | CV (%) | Relative error (%) | CV (%) | Relative error (%) |
| Haloperidol | 1 | 1.5 | −2.0 | 3.1 | −3.7 |
| | 10 | 2.5 | 5.0 | 3.9 | −1.2 |
| | 20 | 1.6 | −1.0 | 1.9 | −2.7 |
| Reduced haloperidol | 1 | 3.1 | −4.0 | 4.0 | −6.2 |
| | 10 | 3.0 | −3.0 | 5.1 | −5.7 |
| | 20 | 1.9 | −3.5 | 2.7 | −4.5 |
| Bromperidol | 1 | 1.3 | −3.0 | 1.0 | −5.8 |
| | 10 | 2.4 | 0.0 | 2.6 | −0.7 |
| | 20 | 1.1 | −1.0 | 1.8 | −2.6 |
| Reduced bromperidol | 1 | 2.3 | −3.0 | 3.2 | −9.5 |
| | 10 | 2.5 | −1.0 | 4.4 | −2.2 |
| | 20 | 1.6 | −4.0 | 1.9 | −2.2 |

Table 4
Time program for the column switching HPLC

| Time after injection (min) | Eluent A or B | |
|---|---|---|
| | Column I | Column II |
| 0.0–10.5 | A | B |
| 10.5–18.0 | B | B |
| 18.0–35.0 | A | B |

### 3.3. Sensitivity

The limit of detection was defined as analyte responses that were at least three times the response compared to blank response. The lowest standard on the calibration curve was defined as the limit of quantification as analyte peaks by which both compounds in blank plasma were identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%. The limits of detection and quantification were 0.5 and 1.0 ng/ml, respectively, for each compound.

### 3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with three different concentrations of four compounds. Intra- and inter-day precisions were assessed by analyzing six quality controls samples at each concentration on the same day and mean values of two quality controls for 6 days, respectively. These extracts underwent the same column-switching procedure. Intra-day coefficient variations (CVs) for haloperidol, reduced haloperidol, bromperidol and reduced bromperidol were <2.5, 3.1, 2.4 and 2.5%, respectively. Inter-day CVs for corresponding compounds were 3.9, 5.1, 2.6 and 4.4%, respectively (Table 4). Accuracy was expressed as mean percent error (relative error) ((measured plasma concentration – spiked concentration in plasma)/spiked concentration in plasma) × 100 (%) of each quality control plasma sample, while precision was quantitated by calculating intra- and inter CV values.

### 3.5. Drug concentrations in human plasma and comparison with other methods

Fig. 4 shows correlation between plasma drug concentrations determined by the HPLC and conventional EIA methods in patients receiving both haloperidol and bromperidol. Because haloperidol determination by conventional EIA methods have about 100% cross-reactivity with bromperidol and 14–50% cross-reactivity with reduced metabolites, plasma drug concentrations deviated far from the values determined by the new HPLC methods. These values by EIA were higher than corresponding values by HPLC. No interfering peaks were observed, despite the fact that many different drugs were co-administered with the test compounds, e.g., levomepromazine, zotepine, risperidone, biperiden, flu-

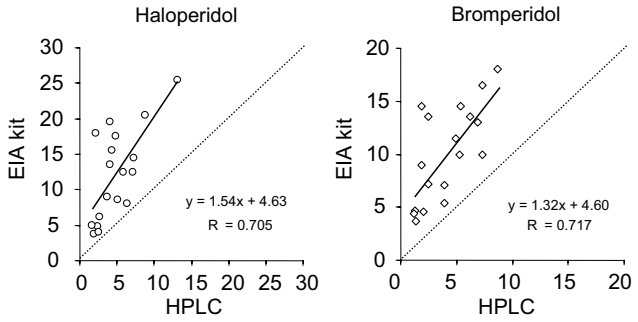


Fig. 4. Correlations of plasma concentrations (ng/ml) of haloperidol and bromperidol between HPLC methods and EIA kits in patients receiving both drugs. The solid line was derived by linear regression of individuals. Dotted line indicates ideal linear regression ($y = x$).

nitrazepam, nitrazepam, brotizolam, diazepam, alprazolam, carbamazepine, lithium and etc.

## 4. Conclusion

The new HPLC procedure described for simultaneous determination of haloperidol, bromperidol and their reduced metabolites is suitable for routine analysis even though it is a little time consuming. Adding perchloric acid resulted in successful baseline separation. Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows measurement of therapeutic concentrations of haloperidol and/or bromperidol, particularly in patients receiving both haloperidol and bromperidol.

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