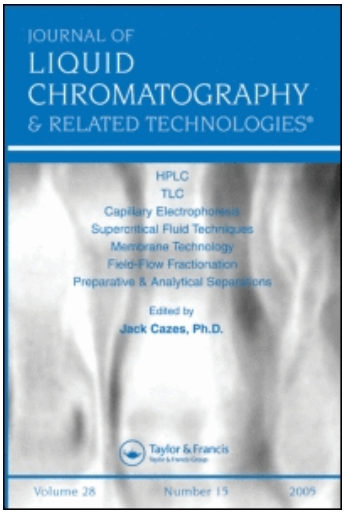

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Simultaneous Determination of *N*-Dealkylated Metabolites of Four Butyrophenone-Type Agents by HPLC with Fluorescence Detection after Pre-Column Derivatization with Dansyl Chloride

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Abstract: The basic metabolites of butyrophenone-type agents are sometimes more neurotoxic than the parent compounds. Recently, we developed a simple high performance liquid chromatographic (HPLC) method coupled with dual ultraviolet detection to determine the *N*-dealkylated basic metabolites, i.e., 1,3-dihydro-1-(1,2,3,6-tetrahydro-4-pyridinyl)-*H*-benzimidazole-2-one, 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, 4-(4-chloro-phenyl)-4-hydroxypiperidine, and 4-(4-bromophenyl)-4-hydroxypiperidine (BPHP), of droperidol, spiperone, haloperidol, and bromperidol, respectively, in phosphate buffered saline (pH 7.4). An HPLC analysis with fluorescence detection was also developed to quantify these metabolites in rat plasma after precolumn derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). In this study, dansyl chloride (Dns-Cl) was used for determination of these metabolites instead of NBD-F. The samples in phosphate buffered saline were mixed with borate buffer (pH 9.5) and Dns-Cl solution in acetonitrile at 60°C for 15 min. HPLC was conducted with a reversed phase (C_{18}) column, eluted with a mixture of methanol—water—acetic acid (650:350:4, v/v/v) at a flow rate of 1.0 mL/min at 25°C. The four Dns-derivatives were well separated from each other in less than 50 min. The calibration curves were linear up to 1 μg/mL, and the lower limits of detection were 0.005 to 0.06 μg/mL. The coefficients of variation were less than 13.1%. Since Dns-Cl is

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a much cheaper labeling agent than NBD-F, the present method is considered to be more suitable and practical for routine determination of these metabolites.

Keywords: Basic metabolite, Butyrophenone type agent, Dansyl chloride, Derivatization

INTRODUCTION

Droperidol, spiperone, haloperidol, and bromperidol are butyrophenone type agents used clinically as pretreatments for systemic anesthetization, and to treat schizophrenia. They are blockers of the dopamine D1 or D2 receptor, and are metabolized to 1,3-dihydro-1-(1,2,3,6-tetrahydro-4-pyridinyl)-2*H*-benzimidazole-2-one (DTP), 1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one (PTS), 4-(4-chloro-phenyl)-4-hydroxypiperidine (CPHP), and 4-(4-bromophenyl)-4-hydroxypiperidine (BPHP), respectively,[1,2] (Figure 1). Ablordeppey et al. reported that CPHP has a delayed and persistent freezing action, which may involve sigma receptors,[3] rather than the dopamine D2 receptor.[4] Similarly, BPHP may be at least partly responsible for the acute dystonia that can be induced by bromperidol treatment.[5,6] These four basic metabolites structurally resemble 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which is a potent neurotoxin

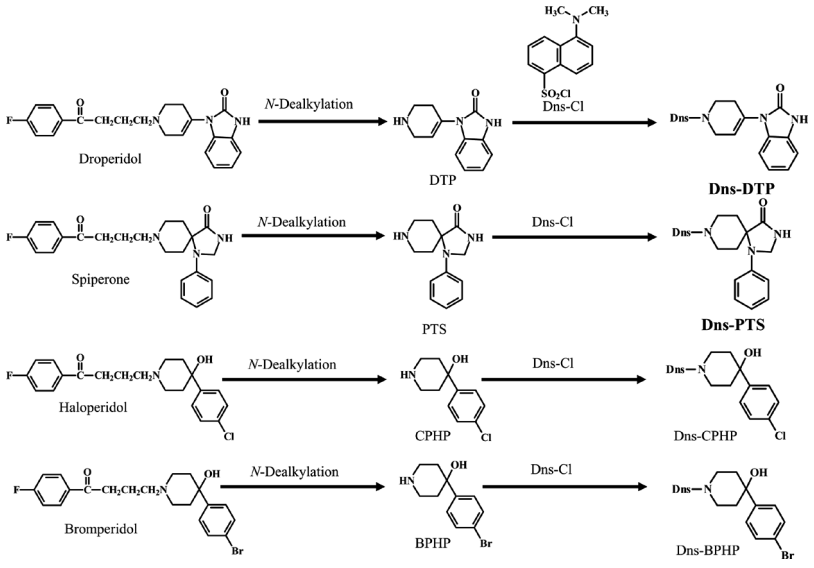

Figure 1. *N*-Dealkylation of butyrophenone-type agents, chemical structures, and scheme of derivatization with Dns-Cl.

capable of causing Parkinson like disease and dyskinesia,[5,7] and they may induce neurotoxicity, so it is important to establish a convenient and practical assay system for their determination.

Several methods have been reported for CPHP determination, using gas chromatography with nitrogen selective detection[3] or with electron capture detection,[8] high performance liquid chromatography HPLC-mass spectrometry,[9–11] and HPLC with UV detection[12–14] or with fluorescence detection (FL) using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F).[15] We recently developed a procedure for the simultaneous determination of the four basic metabolites in rat plasma by HPLC-FL after precolumn derivatization with NBD-F following liquid-liquid extraction with benzene.[16] However, the sensitivity for PTS was poor (0.03 μg/mL), and NBD-F is too expensive for routine use.

Here, in order to overcome these problems, we present an HPLC-FL procedure for the simultaneous separation and quantitation of DTP, PTS, CPHP, and BPHP after precolumn derivatization with dansyl chloride (Dns-Cl), a cheaper labeling agent.

EXPERIMENTAL

Equipment

The HPLC system comprised a model L-6200 pump (Hitachi, Tokyo, Japan), a Rheodyne injection valve (Cotati, CA, USA) with a 50 μL loop and a model RF-10A fluorometer (Shimadzu, Kyoto, Japan) operating at an excitation wavelength of 370 nm and an emission wavelength of 506 nm. The HPLC column (Kanto Chemical, Tokyo, Japan) was 150×4.6 mm i.d. containing 5 μm particles of C_{18} packing material.

Reagents

PTS, CPHP, and BPHP were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dns-Cl and DTP were obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and Acros Organics (Geel, Belgium), respectively. Methanol and general reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Chromatographic Conditions

Quantification of the peaks was performed using a Chromatopac Model C-R3A integrator (Shimadzu). The mobile phase was prepared

by addition of methanol (650 mL) and acetic acid (4 mL) to water (350 mL). The samples were eluted from the column at 25°C at a flow rate of 1.0 mL/min.

Derivatization

Borate buffer (0.1 M) was adjusted to pH 9.5 by addition of NaOH (1 M) and an aliquot (100 μL) was added to phosphate buffered saline (100 μL, 137 mM NaCl, 2 mM, K_2HPO_4, and 8 mM NaH_2PO_4, adjusted to pH 7.4 using NaOH) containing the samples. Dns-Cl solution in acetonitrile (1 mg/mL, 100 μL) was added. The mixture was vortexed, then allowed to stand for 15 min at 60°C, and kept on ice for 1 min. The solution (50 μL) was injected into the HPLC system.

Calibration Curve

A solution of the four basic metabolites (each 1 mg/mL) in 0.01 M HCl was added to phosphate buffered saline (pH 7.4). The concentrations of the basic metabolites used for calibration were 0, 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, and 1 μg/mL.

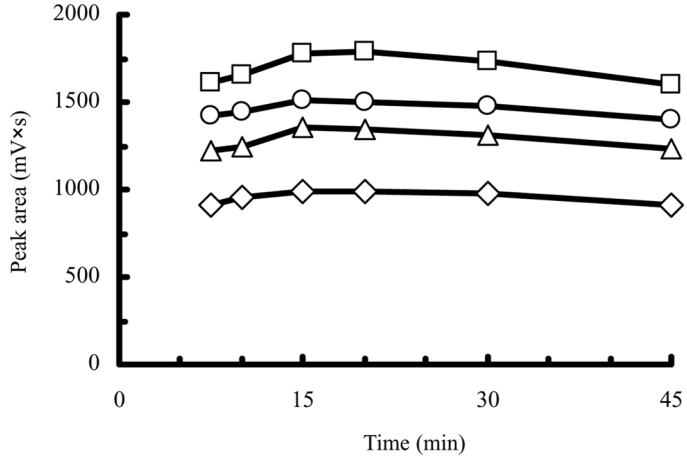

Figure 2. Reaction time courses of derivatization of DTP, PTS, CPHP, and BPHP (each 1 μg/mL) with Dns-Cl. (○) DTP derivative; (Δ) PTS derivative; (□) CPHP derivative; (◇) BPHP derivative.

RESULTS AND DISCUSSION

Derivatization Time Courses of the Four Basic Metabolites with Dns-Cl

For the time course study, the reaction time was set at 7.5, 10, 15, 20, 30, or 45 min. The four basic metabolites (each 1 μg/mL) in phosphate buffered saline were derivatized as described above. The derivatization of all four basic metabolites reached a plateau level within 15 min (Figure 2). At 45 min, the peak areas of the four derivatives tended to be reduced. Thus, the derivatization time of 15 min was chosen as the standard condition.

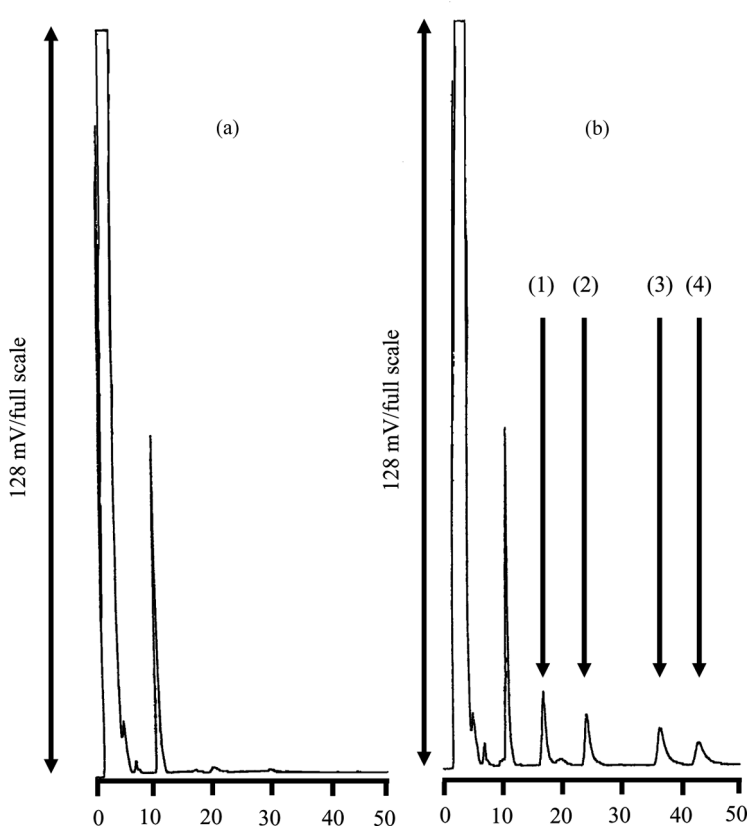

Figure 3. . Chromatograms of DTP, PTS, CPHP, and BPHP derivatives. (a) Blank; (b) Sample spiked with DTP, PTS, CPHP, and BPHP (each 0.5 μg/mL). Peaks: 1 = DTP derivative; 2 = PTS derivative; 3 = CPHP derivative; 4 = BPHP derivative.

Chromatograms

A blank chromatogram and a chromatogram of a standard mixture of the four basic metabolites (each 0.5 μg/mL) are shown in Figure 3. The retention times of the DTP, PTS, CPHP, and BPHP derivatives were 17.3, 25.6, 37.6, and 44.2 min, respectively. The chromatographic run time was 50 min.

Linearity and Lower Limit of Detection

Table 1 summarizes the values of slope, intercept, concentration range, and squared regression coefficient (r^2). The lower limits of detection of DTP, PTS, CPHP, and BPHP were 0.06, 0.008, 0.005, and 0.01 μg/mL (signal-to-noise ratio of 3:1), respectively. The values of the lower limit of quantification, i.e., the lowest concentration on the standard curve that can be measured with acceptable accuracy (relative standard deviation less than 20%, Tables 2 and 3), were 0.1, 0.01, 0.01, and 0.02 μg/mL for DTP, PTS, CPHP, and BPHP, respectively.

Fang et al. determined CPHP by means of GC with electron capture detection, which provided a detection limit of 0.005 μg/mL.[8] The detection limits of CPHP using the HPLC-mass spectrometric procedures of Arinobu et al. were relatively poor (0.075 to 0.3 μg/mL).[9,10] Our previous assays of CPHP by means of HPLC-FL using NBD-F and HPLC-dual UV detection showed detection limits of 0.002 to 0.012 μg/mL.[13,14] Our previous assays of DTP, PTS, and BPHP had detection limits of 0.003 to 0.014, 0.012 to 0.03, and 0.003 to 0.02 μg/mL, respectively.[14–17] Another reported method showed a comparably high sensitivity for CPHP, but slightly lower sensitivity for BPHP. The sensitivity for PTS was 1.5- to 3.8-fold improved compared with our previous results,[14,16] while that for DTP was 4.3- to 20-fold poorer. Further studies may allow the sensitivity for DTP to be improved.

Table 1. Linear correlation parameters

Four basic metabolites	Slope	Intercept	Concentration range	r^2
DTP	1530	+56.34	0.1 to 1 μg/mL	0.9979
PTS	1269	+6.459	0.01 to 1 μg/mL	0.9976
CPHP	1616	+7.849	0.01 to 1 μg/mL	0.9995
BPHP	980.4	−4.990	0.02 to 1 μg/mL	0.9978

Table 2. Intra-day assay reproducibility for determination of DTP, PTS, CPHP, and BPHP

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
DTP			
0.1	0.106 ± 0.009	8.5	106.0
1	0.977 ± 0.048	4.9	97.7
PTS			
0.01	0.0107 ± 0.0009	8.4	107.0
0.1	0.0974 ± 0.0056	5.7	97.4
1	0.999 ± 0.0043	4.3	99.9
CPTH			
0.01	0.0104 ± 0.0009	8.7	104.0
0.1	0.0959 ± 0.0058	6.0	95.9
1	1.07 ± 0.04	3.7	107.0
BPHP			
0.02	0.0204 ± 0.0018	8.8	102.0
0.1	0.106 ± 0.006	5.7	106.0
1	0.998 ± 0.050	5.0	99.8

Table 3. Inter-day assay reproducibility for determination of DTP, PTS, CPHS, and BPHP

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
DTP			
0.1	0.108 ± 0.014	13.0	108.0
1	0.970 ± 0.056	5.8	97.0
PTS			
0.01	0.0107 ± 0.0014	13.1	107.0
0.1	0.0988 ± 0.0069	9.7	98.8
1	0.979 ± 0.092	9.4	97.9
CPTH			
0.01	0.0104 ± 0.0013	12.5	104.0
0.1	0.105 ± 0.010	9.5	105.0
1	1.02 ± 0.086	8.4	102.0
BPHP			
0.02	0.0211 ± 0.0024	11.4	105.5
0.1	0.103 ± 0.009	8.7	103.0
1	0.968 ± 0.074	7.6	96.8

Precision and Accuracy

Precision and accuracy in intra- and inter-day assays of Dns-Cl derivatives are shown in Tables 2 and 3. In the intra-day assay (Table 2), the ranges of standard deviation of the mean of DTP, PTS, CPHP, and BPHP were 4.9 to 8.5, 4.3 to 8.4, 3.7 to 8.7, and 5.0 to 8.8%, respectively. The recoveries of DTP, PTS, CPHP, and BPHP ranged from 95.9 to 107.0%. In the inter-day assay (Table 3), the ranges of standard deviation of the mean of DTP, PTS, CPHP, and BPHP were 5.8 to 13.0, 9.4 to 13.1, 8.4 to 12.5, and 7.6 to 11.4%, respectively. The recoveries of DTP, PTS, CPHP, and BPHP ranged from 96.8 to 108.0%.

CONCLUSION

We used the inexpensive labeling reagent Dns-Cl for HPLC analysis of the basic metabolites of droperidol, spiperone, haloperidol, and bromperidol; the derivatives were well separated from each other, with a total elution time of less than 50 min. The lower limits of detection of PTS, CPHP, and BPHP were in the range of 0.005 to 0.01 μg/mL, while that of DTP was 0.06 μg/mL. This method using Dns-Cl may be suitable for routine determination of these metabolites in patients and experimental animals.

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