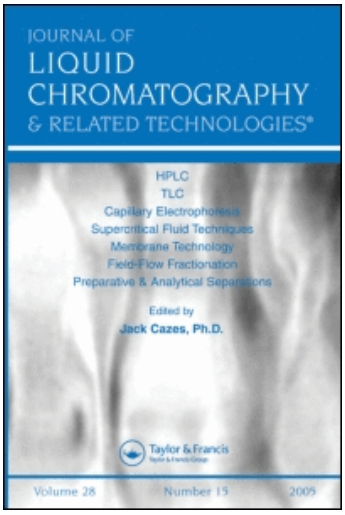

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Yasuhiko Higashi[a]; Masatoshi Sakata[a]; Youichi Fujii[a]
[a] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

To cite this Article Higashi, Yasuhiko , Sakata, Masatoshi and Fujii, Youichi(2006) 'Simultaneous Determination of Four Basic Metabolites Formed from Butyrophenone Type Agents by HPLC with Dual Ultraviolet Detection', Journal of Liquid Chromatography & Related Technologies, 29: 19, 2853 — 2862
To link to this Article: DOI: 10.1080/10826070600959476
URL: http://dx.doi.org/10.1080/10826070600959476

Journal of Liquid Chromatography & Related Technologies®, 29: 2853–2862, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600959476

Simultaneous Determination of Four Basic Metabolites Formed from Butyrophenone Type Agents by HPLC with Dual Ultraviolet Detection

Yasuhiko Higashi, Masatoshi Sakata, and Youichi Fujii
Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

Abstract: Some basic metabolites of butyrophenone-type agents are more neurotoxic than the parent compounds. We have developed a high performance liquid chromatography method with dual ultraviolet detection (HPLC-dual UV method) to quantify simultaneously the four basic metabolites {1,3-dihydro-1-(1,2,3,6-tetrahydro-4-pyridinyl)-2H-benzimidazole-2-one, (DTP) 1-phenyl-1,3,8-triazaspiro[4.5] decan-4-one (PTS), 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP), and 4-(4-bromophenyl)-4-hydroxypiperidine (BPHP)} of droperidol, spiperone, haloperidol, and bromperidol, respectively. DTP, PTS, CPHP, and BPHP were monitored at their UV maxima (278, 247, 220, and 220 nm, respectively) and also at 200 nm. A reversed-phase (C_{18}) column was eluted with a mixture of acetonitrile-water-85% phosphoric acid (80:920:1, v/v/v) at a flow rate of 1.0 mL/min at 25°C. The four basic metabolites were well separated from each other in less than 25 min. The lower limits of detection were 8 to 20 ng/mL with detection at the UV absorption maxima, and 5 to 12 ng/mL with detection at 200 nm. The coefficients of variation for intra-and inter-day assays were less than 13.4%. The recoveries were satisfactory. These results confirm that the HPLC-dual UV method is satisfactory for the simultaneous assay of DTP, PTS, CPHP, and BPHP in phosphate-buffered saline.

Keywords: Butyrophenone-type agent, Basic metabolite, High performance liquid chromatography, Dual ultraviolet detection

Address correspondence to Yasuhiko Higashi, Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan. E-mail: y-higashi@hokuriku-u.ac.jp

INTRODUCTION

Haloperidol and bromperidol are widely used neuroleptic agents that mainly act as blockers of the dopamine D1 or D2 receptor, and are metabolized to 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) and 4-(4-bromophenyl)- 4-hydroxypiperidine (BPHP), respectively.[1,2] Ablordeppey et al. reported that CPHP induces a delayed and persistent freezing action,[3] which may involve sigma receptors, 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] Droperidol and spiperone are also clinically utilized butyrophenone type agents, but so far there is no information on the toxicity of their basic metabolites, 1,3-dihydro-1-(1,2,3,6-tetrahydro-4-pyridinyl)-2H-benzimidazole-2-one (DTP) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (PTS) (Fig. 1).

The above four basic metabolites structurally resemble 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a potent neurotoxin capable of causing Parkinson like disease and dyskinesia.[5,7] Since the four basic metabolites may also induce MPTP like neurotoxicity, it is important to establish an assay system for their determination.

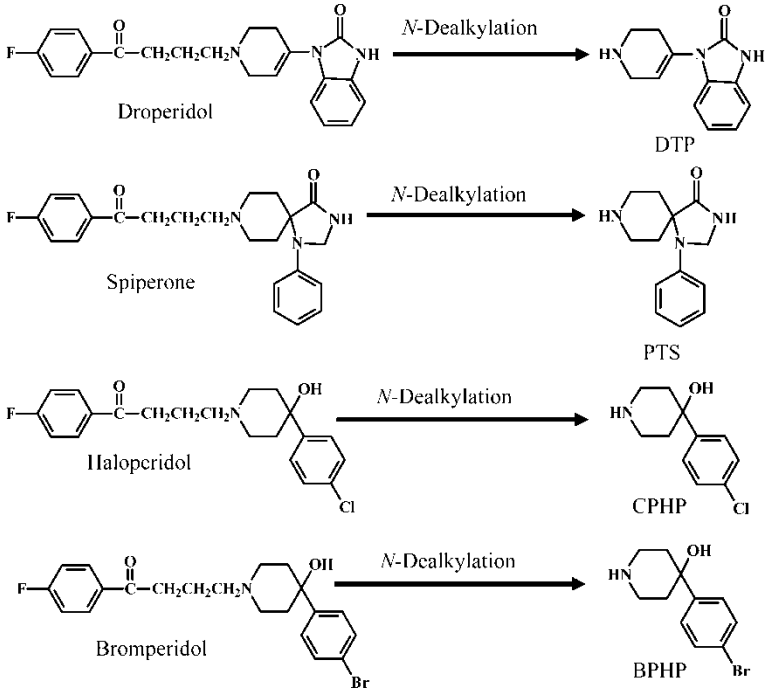

Figure 1. N-Dealkylation of butyrophenone type agents and chemical structures.

Several methods have been reported for CPHP determination using gas chromatography (GC)-nitrogen selective detection,[3] GC-electron capture detection,[8] HPLC-mass spectrometry,[9–11] HPLC-fluorescence detection with 4-fluoro-7-nitro-2,1,3-benzoxadiazole,[12] and HPLC-UV detection.[13,14]

Since HPLC-UV methods are inexpensive and widely utilized, this approach may be preferable for determination of CPHP and the other metabolites. Here, we report an HPLC-UV method for the simultaneous separation and quantitation of DTP, PTS, CPHP, and BPHP in phosphate buffered saline, with UV detection at the respective UV absorption maxima and at 200 nm.

EXPERIMENTAL

Equipment

The HPLC system was comprised of a model LC-10AT pump (Shimadzu, Kyoto, Japan), a Rheodyne injection valve (Cotati, CA, USA) with a 50 μL loop, and a model SPD-10A two channel UV detector (Shimadzu, Kyoto, Japan). Channel 1 was automatically switched between 278 nm for DTP (from 0 min to 8 min), 247 nm for PTS (from 8 min to 15 min), and 220 nm for CPHP and BPHP (from 15 min to 25 min). Channel 2 was operated at 200 nm. The HPLC column (150 × 4.6 mm i.d., Mightysil RP-18 GP, Kanto Chemical, Tokyo, Japan) was packed with 5 μm particles of C_{18} packing material.

Reagents

PTS, CPHP, and BPHP were obtained from Sigma-Aldrich (St. Louis, MO, USA). DTP was purchased from Acros Organics (Geel, Belgium). Other general reagents were supplied by Wako Pure Chemical Industries (Osaka, Japan).

UV Absorption Spectra

The UV absorption spectra of DTP, PTS, CPHP, and BPHP were obtained with a UV-1200 spectrophotometer (Shimadzu). Each compound was dissolved in the mobile phase (5 μg/mL) as described below, and the spectra were determined in the range of 200 to 340 nm.

Chromatographic Conditions

Quantification of the peaks was performed with a Chromatopac Model CR-8A integrator (Shimadzu). The mobile phase was prepared by addition of acetonitrile (80 mL) and 85% phosphoric acid (1 mL) to water (920 mL).

Samples (50 μL) in phosphate buffered saline, containing NaCl (140 mM), K_2HPO_4 (2.5 mM), and NaH_2PO_4 (7.5 mM) at pH 7.4 (adjusted with NaOH), were injected into the HPLC system. The samples were eluted from the column at a flow rate of 1.0 mL/min at 25°C.

RESULTS AND DISCUSSION

UV Absorption Spectra

The UV absorption spectra of DTP, PTS, CPHP, and BPHP are shown in Fig. 2. DTP, PTS, CPHP, and BPHP showed UV absorption maxima at 278, 247, 220, and 220 nm, respectively, and they each also showed marked UV absorption at 200 nm.

Chromatograms

A typical chromatogram of the standard mixture of DTP, PTS, CPHP, and BPHP at the respective UV absorption maxima (A-1) and a blank

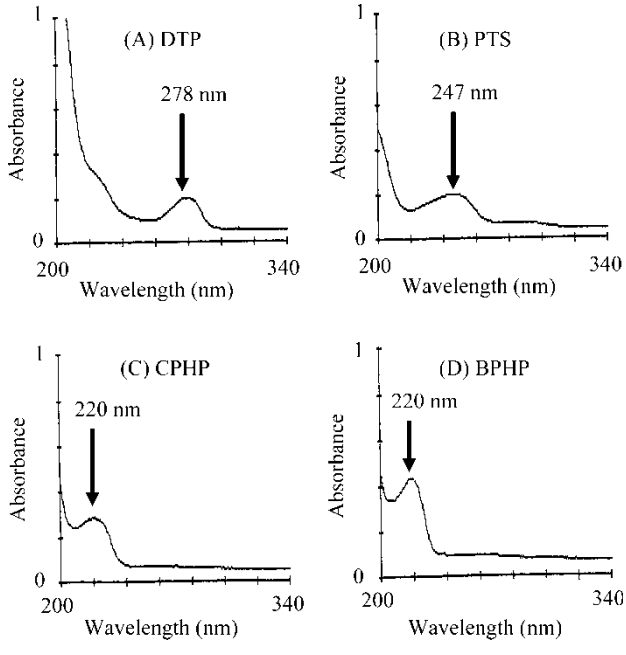

Figure 2. UV absorption soectra of DTP, PTS, CPHP, and BPHP. The concentration of all compounds was 5 mg/mL. The absorption spectrum of the solvent (mobile phase) has been subtracted from all spectra. (A) DTP; (B) PTS; (C) CPHP; (D) BPHP.

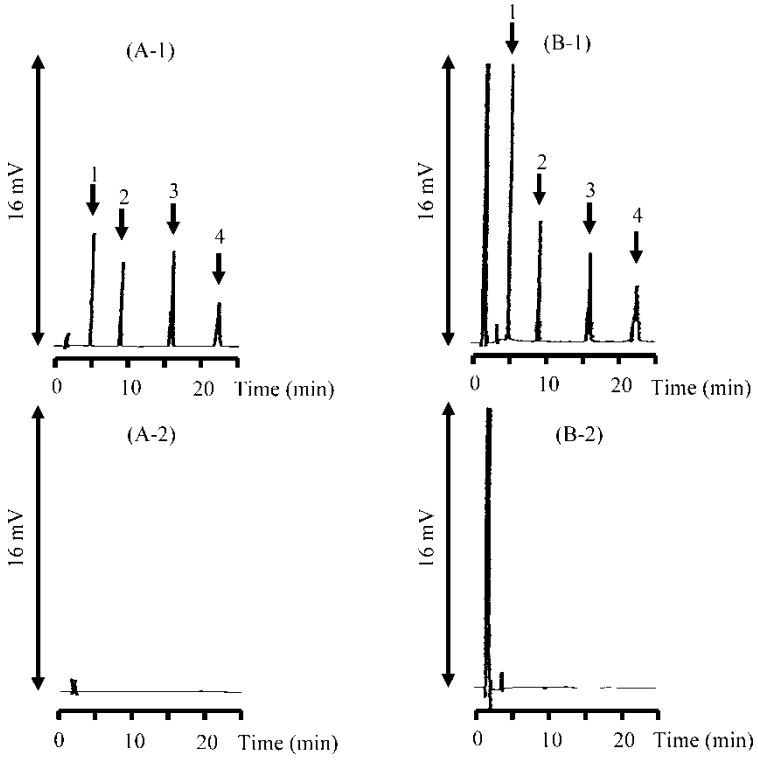

Figure 3. Chromatograms of DTP, PTS, CPHP, and BPHP. The concentration of all compounds was 1 mg/mL in phosphate buffered saline. (A-1) Phosphate buffered saline spiked with DTP, PTS, CPHP, and BPHP, with detection at the respective UV absorption maxima; (A-2) Drug-free phosphate buffered saline with similar detection; (B-1) Phosphate buffered saline spiked with DTP, PTS, CPHP, and BPHP, detected at 200 nm; (B-2) Drug-free phosphate buffered saline, with detection at 200 nm. Peaks: 1 = DTP; 2 = PTS; 3 = CPHP; 4 = BPHP.

chromatogram (A-2) are shown in Fig. 3. A typical chromatogram with detection at 200 nm (B-1) and the corresponding blank chromatogram (B-2) are also shown in Fig. 3. The retention times of DTP, PTS, CPHP, and BPHP in both cases were 4.9, 9.8, 16.4, and 22.5 min, respectively.

Linearity and Lower Limit of Detection

Standard curves of DTP, PTS, CPHP, and BPHP with detection at the absorption maxima were constructed by plotting integrated peak area *vs.* concentration. Linear relationships were found for DTP ($y = 51.61x + 0.426$,

concentrations ranging from 0.02 to 1 μg/mL), PTS ($y = 59.58x - 0.223$, concentrations ranging from 0.02 to 1 μg/mL), CPHP ($y = 108.7x + 0.286$, concentrations ranging from 0.01 to 1 μg/mL), and BPHP ($y = 79.49x - 0.622$, concentrations ranging from 0.05 to 1 μg/mL). The squared regression coefficients (r^2) of DTP, PTS, CPHP, and BPHP were 0.9990, 0.9990, 0.9989, and 0.9998, respectively. The lower limits of detection were 14, 14, 8, and 20 ng/mL (signal-to-noise ratio of 3:1), respectively.

Standard curves of the four basic metabolites with detection at 200 nm were similarly constructed, and linear relationships were obtained: DTP ($y = 197.6x + 0.126$, concentrations ranging from 0.01 to 1 μg/mL), PTS ($y = 79.49x + 1.05$, concentrations ranging from 0.02 to 1 μg/mL), CPHP ($y = 99.15x + 0.530$, concentrations ranging from 0.02 to 1 μg/mL), and BPHP ($y = 90.10x + 1.57$, concentrations ranging from 0.02 to 1 μg/mL). The r^2 values of DTP, PTS, CPHP, and BPHP were 0.9989, 0.9994, 0.9998, and 0.9972, respectively. The lower limits of detection were established at 5, 12, 10, and 10 ng/mL (signal-to-noise ratio of 3:1), respectively.

Fang and Gorrod determined CPHP by means of GC-electron capture detection, which provided a detection limit of 5 ng/mL.[13] Our previous assays of CPHP by means of HPLC-fluorescence detection with 4-fluoro-7-nitro-2,1,3-benzoxadiazole and HPLC-UV had detection limits of 8 and 12 ng/mL, respectively.[12,14] The detection limits of CPHP using the HPLC-mass spectrometric procedures of Arinobu et al. were relatively poor (75 to 300 ng/mL).[9,10] The present HPLC method with UV detection at 220 and 200 nm gave detection limits of 8 and 10 ng/mL for CPHP. Our preliminary study for BPHP determination by HPLC-fluorescence detection with 4-fluoro-7-nitro-2,1,3-benzoxadiazole indicated a detection limit of 3 ng/mL, although the developed method had a somewhat higher detection limit. To our knowledge, no established method is available for DTP and PTS determination.

Precision and Accuracy

Precision and accuracy for intra- and inter-day assays of the compounds, detected at the UV absorption maxima, are shown in Table 1. In the intra-day assay, the coefficients of variation for DTP, PTS, CPHP, and BPHP were in the ranges of 3.7 to 5.2, 4.0 to 6.2, 3.0 to 6.4, and 3.6 to 4.5%, respectively. The recoveries were in the range of 96.5 to 101.0%. In the inter-day assay, the corresponding values were 4.2 to 9.3, 8.9 to 10.7, 7.4 to 9.4, and 8.5 to 11.7%, with recoveries of 94.7 to 107.6%.

The corresponding data obtained with detection at 200 nm are listed in Table 2. In the intra-day assay, the coefficients of variation for DTP, PTS,

Table 1. Intra- and inter-day assay reproducibility for determination of DTP, PTS, CPHP, and BPHP at the respective maximum UV absorption wavelength

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
Intra-day assay			
DTP			
0.02	0.0193 ± 0.0010	5.2	96.5
0.1	0.0994 ± 0.0046	4.6	99.4
1	0.996 ± 0.037	3.7	99.6
PTS			
0.02	0.0194 ± 0.0012	6.2	97.0
0.1	0.0982 ± 0.047	4.8	98.2
1	0.998 ± 0.040	4.0	99.8
CPHP			
0.01	0.00970 ± 0.00062	6.4	97.0
0.1	0.0971 ± 0.0033	3.4	97.1
1	1.01 ± 0.03	3.0	101.0
BPHP			
0.05	0.0490 ± 0.0022	4.5	98.0
0.1	0.0981 ± 0.0041	4.2	98.1
1	0.984 ± 0.035	3.6	98.4
Inter-day assay			
DTP			
0.02	0.0193 ± 0.0018	9.3	96.5
0.1	0.0996 ± 0.0042	4.2	99.6
1	0.994 ± 0.062	6.2	99.4
PTS			
0.02	0.0206 ± 0.0022	10.7	103.0
0.1	0.0961 ± 0.0086	8.9	96.1
1	0.962 ± 0.096	10.2	96.2
CPHP			
0.01	0.0106 ± 0.0010	9.4	106.0
0.1	0.0947 ± 0.0070	7.4	94.7
1	1.02 ± 0.09	8.8	102.0
BPHP			
0.05	0.0538 ± 0.0063	11.7	107.6
0.1	0.0961 ± 0.0082	8.5	96.1
1	0.984 ± 0.095	9.7	98.4

CPHP, and BPHP were in the ranges of 4.0 to 5.4, 3.2 to 5.1, 3.9 to 4.7, and 4.3 to 5.0%, respectively, with recoveries of 96.0 to 104.0%. In the inter-day assay, the corresponding values were 6.8 to 8.2, 8.7 to 13.3, 9.6 to 11.0, and 8.9 to 13.4%, with recoveries of 95.5 to 104.0%.

Table 2. Intra- and inter-day assay reproducibility for determination of DTP, PTS, CPHP, and BPHP at 200 nm

Concentration (μg/mL)	Measured (μg/mL) (Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
Intra-day assay			
DTP			
0.01	0.00961 ± 0.00052	5.4	96.1
0.1	0.0982 ± 0.0040	4.1	98.2
1	1.01 ± 0.04	4.0	101.0
PTS			
0.02	0.0197 ± 0.0010	5.1	98.5
0.1	0.0986 ± 0.0035	3.5	98.6
1	0.993 ± 0.032	3.2	99.3
CPHP			
0.02	0.0192 ± 0.0009	4.7	96.0
0.1	0.0971 ± 0.0042	4.3	97.1
1	1.02 ± 0.04	3.9	102.0
BPHP			
0.02	0.0193 ± 0.0009	4.7	96.5
0.1	0.0991 ± 0.0043	4.3	99.1
1	1.01 ± 0.05	5.0	101.0
Inter-day assay			
DTP			
0.01	0.00986 ± 0.00081	8.2	98.6
0.1	0.0995 ± 0.0072	7.2	99.5
1	1.03 ± 0.07	6.8	103.0
PTS			
0.02	0.0195 ± 0.0026	13.3	97.5
0.1	0.103 ± 0.009	8.7	103.0
1	0.986 ± 0.098	9.9	98.6
CPHP			
0.02	0.0191 ± 0.0021	11.0	95.5
0.1	0.102 ± 0.010	9.8	102.0
1	1.04 ± 0.10	9.6	104.0
BPHP			
0.02	0.0202 ± 0.0027	13.4	101.6
0.1	0.101 ± 0.009	8.9	101.0
1	1.03 ± 0.10	9.7	103.0

CONCLUSION

We have established an HPLC-dual UV method for the simultaneous determination of DTP, PTS, CPHP, and BPHP, which are metabolites of droperidol,

spiperone, haloperidol, and bromperidol, respectively, in phosphate-buffered saline. Although further studies are needed to establish what pretreatment would be necessary to separate the metabolites from biological matrices, our assay system is expected to be useful for toxicological analysis and monitoring the levels of the metabolites.

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Received June 15, 2006
Accepted July 22, 2006
Manuscript 6905