J. Cosmet. Sci., 66, 129–137 (March/April 2015)

Simple determination of *o*-phenylphenol in skin lotion by high-performance liquid chromatography coupled with fluorescence detection after pre-column derivatization with 4-(*N*-chloroformylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole

YASUHIKO HIGASHI and KAZUNORI KONNO, *Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-machi, Kanazawa 920-1181, Japan.*

Accepted for publication February 15, 2015.

Synopsis

o-Phenylphenol (OPP) in skin lotion was quantitated by high-performance liquid chromatography coupled with fluorescence detection after pre-column derivatization with 4-(*N*-chloroformylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-COCl) in borate buffer (pH 8.5) at room temperature for 2 min. The column [150 mm × 3.0 mm internal diameter (i.d.)], which contained 5 μm particles of C_{18} packing material, was eluted at room temperature (flow rate: 0.5 ml/min) with mobile phase prepared by addition of acetonitrile (550 ml) to 450 ml of Milli-Q water containing trifluoroacetic acid (0.1 v/v%). 2-Hydroxyfluorene was used as an internal standard. The retention times of NBD-CO-OPP and NBD-CO-IS derivatives were 16.2 and 22.2 min, respectively. The calibration plot was linear in the range of 0.01–0.2 μg/ml with an r^2 value of 0.9960, and the lower limit of detection was 0.003 μg/ml (at a signal-to-noise ratio of 3:1; absolute amount of 12 pg/20 μl injection). The coefficient of variation was less than 8.8%. Contents of OPP in three skin lotions were determined with the present system, and the recovery from spiked samples was satisfactory.

INTRODUCTION

o-Phenylphenol (OPP) has antibacterial and antiviral activities, and is widely used in households, industry, and hospitals to disinfect surfaces, and as a preservative in cosmetics, plastics, etc. (1,2). Although OPP is an irritant for skin and mucous membranes, it exhibited low acute toxicity in animal experiments (3). Dermal administration of OPP promoted skin carcinogenesis in CD-1 female mice initiated with 7,12-dimethylbenz[*a*]anthracene (4).

The Ministry of Health, Labour and Welfare in Japan recommends that the upper limit level of OPP in cosmetics should be 0.30 g/100 g for mucous membranes and for skin areas that are not washed after application, although the level has not been decided for the

Address all correspondence to Yasuhiko Higashi at y-higashi@hokuriku-u.ac.jp.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

case of cosmetics where the skin is washed after application (5). On the other hand, the Japanese government approved the use of OPP as a food additive for citrus fruits in 1977 with the permitted maximum residue level of 10 ppm in whole fruits (6,7). The World Health Organization's view on the toxicity of OPP is as follows (8): "A health-based value of 1 mg/l can be calculated for OPP on the basis of an ADI of 0.4 mg/kg of body weight, based on a NOAEL of 39 mg/kg of body weight per day in a 2-year toxicity study for decreased body weight gain and hyperplasia of the urinary bladder and carcinogenicity of the urinary bladder in male rats, using an uncertainty factor of 100. Because of its low toxicity, however, the health-based value derived for OPP is much higher than OPP concentrations likely to be found in drinking-water. Under usual conditions, therefore, the presence of OPP in drinking-water is unlikely to represent a hazard to human health."

Analysis of OPP in grapefruit juice has been performed by high-performance liquid chromatography with ultraviolet absorption detection (HPLC—UV) after pre-column labeling with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) (9). While this system is simple, it shows poor sensitivity. Yang *et al.* (7) developed a highly sensitive method of OPP determination by HPLC with electrochemical detection, using a microbore column; this afforded a detection limit of 3.4 pg. Gas chromatography—mass spectrometric (GC—MS) methods for determination of OPP after derivatization with pentafluorobenzoyl bromide and ferrocenecarboxylic acid chloride have been applied to beer and citrus fruit samples, respectively (1,2). Blasco *et al.* (10) used liquid chromatography (LC)—atmospheric pressure chemical ionization MS for OPP determination in fruits and vegetables. However, MS or electrochemical detection requires expensive equipment. Instead, a simple and inexpensive method is desirable for routine OPP analysis.

NBD-F has been used as a fluorescence labeling agent of primary and secondary amino groups for HPLC—fluorescence detection (11—15). The NBD heterocyclic ring is strongly fluorescent, but NBD-labeling at the phenolic hydroxyl group of *N*-acetyltyrosine, chlorophenols, eugenol or OPP does not afford a fluorescent derivative, so NBD-F has been used for labeling of these compounds in combination with UV detection (9,16—19). Here, we set out to develop a simple, more sensitive HPLC-fluorescence analysis for determination of OPP in skin lotion by means of pre-column derivatization with 4-(*N*-chloroformylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-COCl), which is expected to be available as a fluorescence labeling agent for the phenolic hydroxyl group of OPP. The derivatization scheme is shown in Figure 1.

EXPERIMENTAL

APPARATUS

The HPLC system comprised a model L-6200 pump (Hitachi, Tokyo, Japan), a Rheodyne injection valve (Cotati, CA) with a 20-μl loop and a model RF-10A fluorometer (Shimadzu, Kyoto, Japan) operating at an excitation wavelength of 470 nm and an emission wavelength of 540 nm. The HPLC column (ODS-4; GL Science, Tokyo, Japan) was 150 mm × 3.0 mm i.d. in size, and contained 5 μm particles of C_{18} packing material. Quantification of peaks was performed using a Chromatopac Model C-R3A integrator (Shimadzu, Kyoto, Japan). The mobile phase was prepared by the addition of acetonitrile (550 ml) to 450 ml of Milli-Q water containing trifluoroacetic acid

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

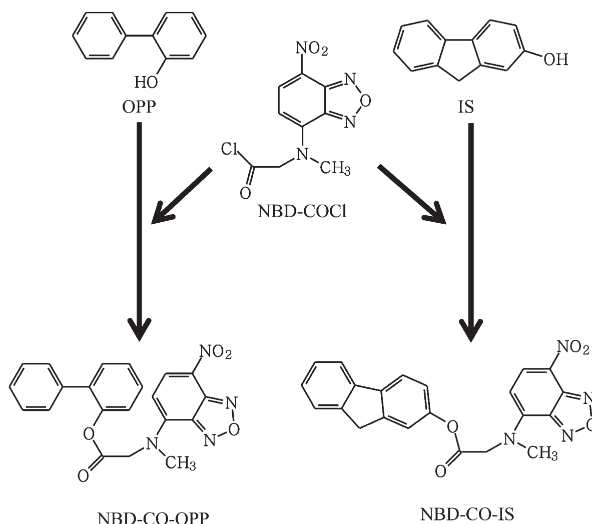

Figure 1. Derivatization of OPP and IS with NBD-COCl.

(0.1 v/v%). The samples were eluted from the column at room temperature at a flow rate of 0.5 ml/min.

REAGENTS

OPP, 2-hydroxyfluorene as an internal standard (IS), NBD-COCl, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, isopropyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate, isobutyl 4-hydroxybenzoate, and benzyl 4-hydroxybenzoate were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Paraben-free skin lotions (A, B, and C) were purchased from a market in Kanazawa city, Ishikawa Prefecture, Japan. Although OPP was stated to be present on the container labels of skin lotions A and B, the concentration was not given. Other general reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

PROCEDURES

Derivatization. Ultrapure water was from a Milli-Q water purification system (Simplicity® UV; Millipore Corporation, Bedford, MA). A standard solution of OPP (2 mg) in methanol (10 ml) was prepared and stocked at 4°C. Working standard solutions (0, 0.01, 0.02, 0.05, 0.1, and 0.2 μg/ml) were prepared by dilution with 10% methanol. Borate buffer (0.1 M) was adjusted to pH 8.5 by the addition of NaOH. Borate buffer (100 μl) was added to a mixture of a diluted standard sample (100 μl) and IS in 0.1% acetonitrile solution (100 μl, 0.1 μg/ml), then NBD-COCl solution in acetonitrile (100 μl, 2 mg/ml) was added. After reaction for 2 min at room temperature, saturated L-aspartate solution (100 μl, filtrate of 5 mg/ml of L-aspartate suspension) was added to stop the reaction, and an aliquot (20 μl) of the solution was injected into the HPLC system.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Sample preparation and addition-recovery tests. Three tested skin lotions (A, 5.0 mg; B, 100 mg; C, 200 mg) were each diluted to 50 ml with 10% methanol, derivatized, and analyzed as described above. Addition-recovery tests were carried out to assess the accuracy of the method by spiking skin lotion samples with OPP (0.005 mg for A, 0.003 mg for B, 0.003 mg for C). An aliquot of 100 μl was analyzed and the OPP concentration in each sample was determined. Recovery was calculated as follows:

$$\text{Recovery } (\%) = \left(\frac{(\text{Total amount after spiking}) - (\text{Spiked amount})}{\text{Original amount}}\right) \times 100$$

RESULTS AND DISCUSSION

DERIVATIZATION OF OPP WITH NBD-COCL

For the time-course study, the reaction time was set at 1, 2, 3, 5, or 10 min at room temperature at various pH values. OPP (100 μl, 0.1 μg/ml), IS solution (100 μl, 0.1 μg/ml), borate buffer (100 μl, various pH values), and NBD-COCl (100 μl, 2 mg/ml) were mixed as described in Experimental. The reaction of OPP at pH 8.5 was faster than under the other pH conditions examined, and the derivatization of OPP reached a maximum at 2 min, but subsequently declined somewhat (Figure 2A). The peak area ratio of NBD-CO-OPP derivative to NBD-CO-IS derivative was most stable at pH 8.5 and 9.0 (Figure 2B). Thus, the derivatization time of 2 min at pH 8.5 was selected.

CHROMATOGRAMS

Figure 3 shows typical chromatograms obtained from (a) blank spiked with IS and (b) standard sample (0.1 μg/ml) spiked with IS. The retention times of NBD-CO-OPP and

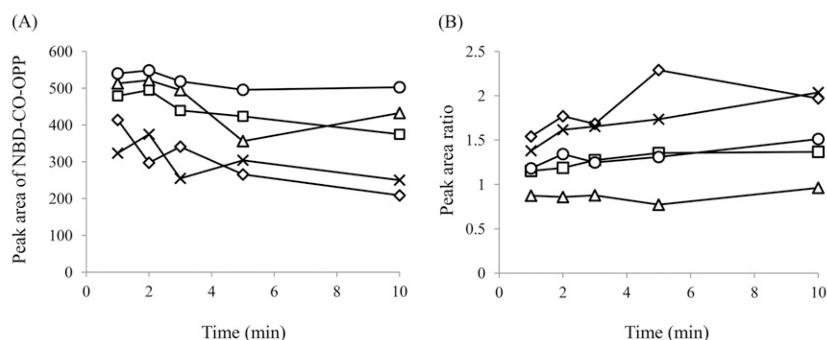

Figure 2A. Time courses of formation of NBD-CO-OPP derivative. Standard samples (0.1 μg/mL) were reacted with NBD-COCl in borate buffer at pH 8.0 to 10.0 at room temperature. (△), pH 8.0 ; (○), pH 8.5; (□), pH 9.0; (◇), pH 9.5; (×), pH 10.0. Data are expressed as mean values of two experiments.
Figure 2B. Time courses of peak area ratio for formation of NBD-CO-OPP and NBD-CO-IS derivatives. Standard sample (0.1 μg/mL) and IS solution (0.1 μg/mL) were reacted with NBD-COCl in borate buffer at pH 8.0 to 10.0 at room temperature. (△), pH 8.0 ; (○), pH 8.5; (□), pH 9.0; (◇), pH 9.5; (×), pH 10.0. Data are expressed as mean values of two experiments.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

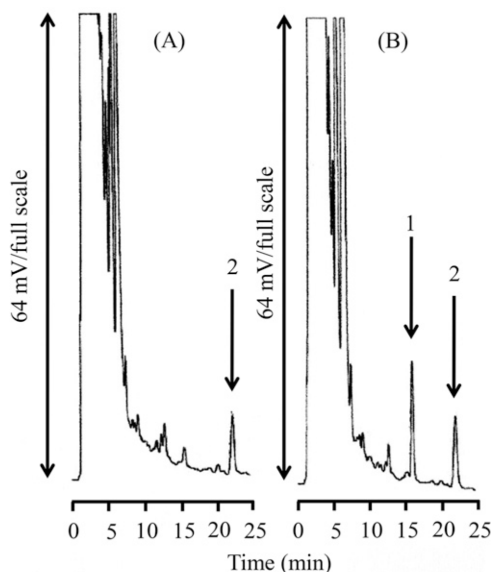

Figure 3. Typical chromatograms of blank spiked with IS (A) and standard sample spiked with IS (B) after derivatization with NBD-COCl. Samples were reacted with NBD-COCl for 2 min at pH 8.5 at room temperature. Compounds: OPP, 0.4 ng/20 μl injection volume; IS, 0.4 ng/20 μl injection volume. Retention times: 16.2 min, NBD-CO-OPP (1); 22.2 min, NBD-CO-IS (2). Chromatogram (A) shows no peak of NBD-CO-OPP (1).

NBD-CO-IS derivatives were 16.2 and 22.2 min, respectively. The running time was 25 min.

METHOD VALIDATION

Linearity. A standard curve was constructed by plotting integrated peak area ratio versus concentration of OPP. The plot was linear (y = 6.297×–0.0217) in the range of 0.01–0.2 μg/ml with an r^2 value of 0.9960.

Sensitivity. The values of the lower limits of quantification and detection were 10 ng/ml (absolute amount of 40 pg/20 μl injection, signal-to-noise ratio of 10:1) and 3 ng/ml (absolute amount of 12 pg/20 μl injection, signal-to-noise ratio of 3:1), respectively. As shown in Table I, the sensitivity of our method (3 ng/ml, absolute amount of 12 pg) was no less than those of various previously reported methods (range of 3.4–350 pg as absolute amount) (6,7,9,10,19–21).

PRECISION AND ACCURACY

Precision and accuracy for intra-day and inter-day assays of OPP are shown in Table II. In the intra-day assay, the range of standard deviation was within 4.5–6.4% of the mean, and recoveries were within the range of 91.4–96.0%. In the inter-day assay, the range of standard deviation was within 5.3–8.8% of the mean, and recoveries were within the range of 89.4–94.5%.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Table I
Sensitivity of various methods for determination of OPP

Method	Limit of detection		Reference
	Concentration (ng/ml)	Absolute amount (pg)	
μHPLC with electrochemical detection	0.68	3.4	(7)
HPLC-UV	17.6	350	(6)
HPLC-UV	24	300	(9)
LC-MS	10	200	(10)
LC-MS/MS	0.1	Not described	(20)
GC-MS	9	Not described	(21)
HPLC-fluorescence detection	10	100	(19)
HPLC-fluorescence detection	3	12	This paper

INTERFERENCE

Cosmetics generally contain parabens as a preservative, because of their antimicrobial activities, relatively low toxicity to human, nonvolatility and other properties (22–24). Therefore, interference with the detection of NBD-CO-OPP or NBD-CO-IS derivatives by seven parabens (methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, isopropyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate, isobutyl 4-hydroxybenzoate, benzyl 4-hydroxybenzoate, each 1 μg/ml in 1% acetonitrile) was investigated. Derivatization was performed as described in Procedures. The relative retention times of seven NBD-CO-paraben derivatives were within 0.34–1.00 (Table III). Only NBD-CO-butyl 4-hydroxybenzoate derivative overlapped with NBD-CO-IS derivative in the chromatogram. Therefore, the present assay is not appropriate for analysis of cosmetics containing butyl 4-hydroxybenzoate. For cosmetics that include butyl 4-hydroxybenzoate as an ingredient, it would be necessary to select a different IS or to conduct the assay without IS. Further studies are needed on this point.

ANALYSIS OF OPP IN THREE SKIN LOTIONS

We selected three skin lotions (A, B, and C) that were all labeled on the bottles as paraben-free. In preliminary tests, no peak that would overlap with NBD-CO-IS was observed in

Table II
Intra- and inter-day assay reproducibility for determination of OPP

	Concentration (μg/ml)	Measured (μg/ml, Mean ± S.D., $n=5$)	C.V. (%)	Recovery(%)
Intra-day				
OPP	0.01	0.00914 ± 0.00062	6.8	91.4
	0.05	0.0462 ± 0.0026	5.6	92.4
	0.2	0.192 ± 0.008	4.2	96.0
Inter-day				
OPP	0.01	0.00894 ± 0.00079	8.8	89.4
	0.05	0.047 ± 0.0032	6.8	94.2
	0.2	0.189 ± 0.010	5.3	94.5

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Table III
Relative retention times of paraben derivatives tested for interference with NBD-CO-OPP and NBD-CO-IS

Compounds	Relative retention time
Methyl 4-hydroxybenzoate	0.34
Ethyl 4-hydroxybenzoate	0.47
Isopropyl 4-hydroxybenzoate	0.64
Propyl 4-hydroxybenzoate	0.67
OPP	0.73
Benzyl 4-hydroxybenzoate	0.93
Isobutyl 4-hydroxybenzoate	0.94
Butyl 4-hydroxybenzoate	0.99
IS	1.00

chromatograms of the three skin lotions, indicating that these skin lotions did not contain butyl 4-hydroxybenzoate or other interfering contaminants. Figure 4 shows typical chromatograms obtained from three samples of the skin lotions. The peak of NBD-CO-OPP was detected in A and B. On the other hand, no NBD-CO-OPP peak was observed in C.

As shown in Table IV, the concentrations of OPP in the three skin lotions (A, B, and C) were found to be 1.07±0.06 mg/g (mean ± SD, *n* = 5, range, 0.996–1.14 mg/g,), 18.8 ± 1.3 μg/g (mean ± SD, *n* = 5, range, 17.5–20.2 μg/g), and ND (not detectable, less than 5.0 μg/g, *n* = 5), respectively. Recovery values of spiked OPP from the lotions A, B, and C were 88.7±5.4% (mean ± SD, *n* = 5, range, 81.2–94.3%). 88.2 ± 4.2% (mean ± SD, *n* = 5, range, 83.6–92.6%), and 88.9 ± 3.5% (mean ± SD, *n* = 5, range, 85.0–93.8%), respectively. The results of addition–recovery tests in the present system were satisfactory.

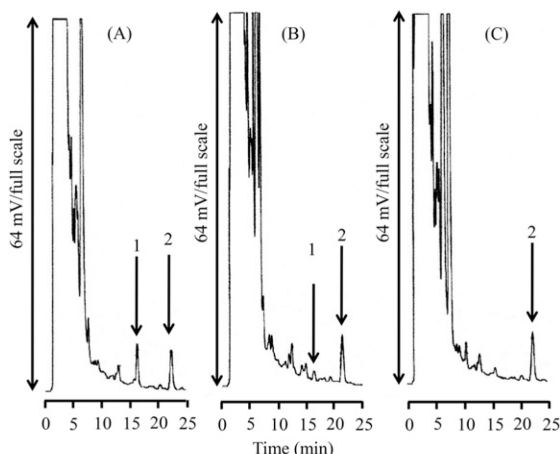

Figure 4. Typical chromatogram of diluted skin lotion samples (A, B, and C) after derivatization with NBD-COCl.Skin lotion was diluted with water as described in EXPERIMENTAL, and mixtures of diluted samples and IS solution were reacted with NBD-COCl for 2 min at pH 8.5 at room temperature. Retention times: 16.2 min, NBD-CO-OPP (1); 22.2 min, NBD-CO-IS (2). Sample (C) showed no peak of NBD-CO-OPP derivative (1).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Table IV
Levels of OPP in skin lotions and relative recovery values

Skin lotion	Concentration (mean ± S.D., $n=5$)	Relative recovecy (%,mean ± S.D., $n=5$)
A	1.07 ± 0.06 mg/g	87.0 ± 3.6
B	18.8 ± 1.3 μg/g	88.7 ± 5.4
C	N.D.	88.9 ± 3.5

N.D., not determined (below the lower limit of quantification).

CONCLUSION

We have developed an HPLC–fluorescence detection analysis for determination of OPP in paraben-free skin lotions by using NBD-COCl as a pre-column fluorescence labeling reagent. This method is rapid, simple, convenient, inexpensive, and should be suitable for routine quality assessment of OPP in cosmetics. Two tested skin lotions were found to contain OPP at mean concentrations of 1.07±0.06 mg/g and 18.8±1.3 μg/g using the present system. The OPP level in the other lotion was below the limit of detection. Previous reports have dealt with determination of OPP levels in various sample matrixes, including fruits, serum, and environmental water (6,7,9,10,19–21). Our present method should be suitable for routine assessment of OPP levels, not only in cosmetics but also in those samples.

REFERENCES

(1) M. Davoren and A. M. Fogarty, In vitro cytotoxicity assessment of the biocidal agents sodium *o*-phenylphenol, sodium *o*-benzyl-*p*-chlorophenol, and sodium *p*-tertiary amylphenol using established fish cell lines, *Toxicol In Vitro.*, **20**, 1190–1201 (2006).
(2) N. Kolbe and J.T. Andersson, Simple and sensitive determination of *o*-phenylphenol in citrus fruits using gas chromatography with atomic emission or mass spectrometric detection, *J. Agric. Food Chem.*, **54**, 5736–5741 (2006).
(3) E. M. Bomhard, S. Y. Brendler-Schwaab, A. Freyberger, B. A. Herbold, K. H. Leser, and M. Richter, *o*-Phenylphenol and its sodium and potassium salts: A toxicological assessment, *Crit. Rev. Toxicol.*, **32**, 551–626 (2002).
(4) M. Takahashi, H. Sato, K. Toyoda, F. Furukawa, K. Imaida, R. Hasegawa, and Y. Hayashi, Sodium *o*-phenylphenate (OPP-Na) promotes skin carcinogenesis in CD-1 female mice initiated with 7,12-dimethylbenz[*a*]anthracene, *Carcinogenesis*, **10**, 1163–1167 (1989).
(5) Ministry of Health, Labour and Welfare. http://www.mhlw.go.jp/file/06-Seisakujouhou-11120000-Iyakushokuhinkyoku/keshouhin-standard.pdf. (2000), pp. 1–11.
(6) R. D. Thompson, Determination of phenolic disinfectant agents in commercial formulation by liquid chromatography, *J. AOAC Int.*, **84**, 815–822 (2001).
(7) L. Yang, A. Kotani, H. Hakamata, and F. Kusu, Determination of *ortho*-phenylphenol residues in lemon rind by high-performance liquid chromatography with electrochemical detection using a microbore column, *Anal. Sci.*, **20**, 199–203 (2004).
(8) World Health Organization. *Guidelines for drinking-water quality incorporating first addendum. Recommendations*, *3rd Ed*. (World Health Organization, 2006), Vol. 1, pp. 427–428.
(9) Y. Higashi and Y. Fujii, Determination of three phenylphenols in grapefruit juice by HPLC after pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole, *J. Anal. Chem.* (2014); In press.
(10) C. Blasco, Y. Picó, J. Mañes, and G. Font, Determination of fungicide residues in fruits and vegetables by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J. Chromatogr. A*, **947**, 227–235 (2002).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

(11) K. Imai, Analytical chemical studies on high-performance recognition and detection of bio-molecules in life, *Yakugaku Zasshi*, **123**, 901–917 (2003).
(12) T. Fukushima, J. Kawai, K. Imai, and T. Toyo'oka, Simultaneous determination of D- and L-serine in rat brain microdialysis sample using a column-switching HPLC with fluorimetric detection, *Biomed. Chromatogr.*, **18**, 813–819 (2004).
(13) Y. Higashi, S. Nakamura, H. Matsumura, and Y. Fujii, Simultaneous liquid chromatographic assay of amantadine and its four related compounds in phosphate-buffered saline using 4-fluoro-7-nitro-2,1,3-benzoxadiazole as a fluorescent derivatization reagent, *Biomed. Chromatogr.*, **20**, 423–428 (2006).
(14) Y. Higashi, M. Sakata, and Y. Fujii, Simultaneous determination of the *N*-dealkylated metabolites of four butyrophenone-type agents in rat plasma by HPLC with fluorescence detection after precolumn derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole, *J. Liq. Chromatogr. Rel. Technol.*, **30**, 2747–2754 (2007).
(15) Y. Higashi, R. Gao, and Y. Fujii, Determination of fluoxetine and norfluoxetine in human serum and urine by HPLC using a Cholester column with fluorescence detection, *J. Liq. Chromatogr. Rel. Technol.*, **32**, 1141–1151 (2009).
(16) T. Toyo'oka, T. Mantani, and M. Kato, Characterization of labelling and de-labelling reagents for detection and recovery of tyrosine residue in peptide, *Biomed. Chromatogr.*, **17**, 133–142 (2003).
(17) Y. Higashi and Y. Fujii, HPLC-UV analysis of phenol and chlorophenols in water after pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole, *J. Liq. Chromatogr. Rel. Technol.*, **32**, 2372–2383 (2009).
(18) Y. Higashi and Y. Fujii, HPLC-UV analysis of eugenol in clove and cinnamon oils after pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole, *J. Liq. Chromatogr. Rel. Technol.*, **34**, 18–25 (2011).
(19) Y. Yamazaki and T. Ninomiya, Determination of benomyl, diphenyl, *o*-phenylphenol, thiabendazole, chlorpyrifos, methidathion, and methyl parathion in oranges by solid-phase extraction, liquid chromatography, and gas chromatography, *J. AOAC Int.*, **82**, 1474–1478 (1999).
(20) L. Yu, R. Schoen, A. Dunkin, M. Firman, H. Cushman, and A. Fontanilla, Determination of *o*-phenylphenol, diphenylamine, and propargite pesticide residues in selected fruits and vegetables by gas chromatography/mass spectrometry, *J. AOAC Int.*, **80**, 651–656 (1997).
(21) X. Ye, L. J. Tao, L. L. Needham, and A. M. Calafat, Automated on-line column-switching HPLC-MS/MS method for measuring environmental phenols and parabens in serum, *Talanta*, **76**, 865–871 (2008).
(22) J. Q. Huang, C. C. Hu, and T. C. Chiu, Determination of seven preservatives in cosmetic products by micellar electrokinetic chromatography, *Int J Cosmet Sci.*, **35**, 346–353 (2013).
(23) C. Charnock and T. Finsrud, Combining esters of para-hydroxy benzoic acid (parabens) to achieve increased antimicrobial activity, *J. Clin. Pharm. Ther.*, **32**, 567–572 (2007).
(24) P. D. Darbre and P. W. Harvey, Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, *J Appl Toxicol.*, **28**, 561–578 (2008).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)