SIMPLE ANALYSIS OF ACETAMINOPHEN IN HUMAN PLASMA BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

Jpn J Forensic Toxicol, Vol 22, No. 1, 2004

Original

SIMPLE ANALYSIS OF ACETAMINOPHEN IN HUMAN PLASMA BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

Makoto HAMAJIMA[a], Saori ISHIDA[a], Yukari HIRATA[a], Kouichi HIRATA[a], Kanako WATANABE [b], Hiroshi SENO [c] and Akira ISHII [a*]

[a] *Department of Legal Medicine, Fujita Health University School of Medicine, 1-98 Kutsukake-cho, Toyoake 470-1192, Japan*
[b] *Department of Legal Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan*
[c] *Department of Legal Medicine, Aichi Medical University, 21 Karimata, Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan*

Received July 23, 2003
Accepted December 17, 2003

固相マイクロ抽出法を用いた血漿中アセトアミノフェンの簡便GC分析

浜島　誠[a]、石田沙織[a]、平田ゆかり[a]、平田公一[a]、渡部加奈子[b]、妹尾　洋[c]、石井　晃[a*]

[a] 藤田保健衛生大学医学部法医学教室　〒470-1192 愛知県豊明市沓掛町1-98
[b] 浜松医科大学法医学教室　〒431-3192 静岡県浜松市半田山 1-20-1
[c] 愛知医科大学法医学教室　〒480-1195 愛知県愛知郡長久手町岩作雁又21

Summary

We have developed a simple method for extraction of acetaminophen (paracetamol) in human plasma by solid-phase microextraction (SPME) using 2-acetamidophenol as internal standard (IS). After heating a vial containing a plasma sample with acetaminophen and IS at 65℃ for 30 min in the presence of Na_2SO_4 and $NaHCO_3$ solution, a polyacrylate–coated fiber was exposed to the liquid-phase to allow adsorption of acetaminophen and IS. The fiber needle was then injected into a capillary gas chromatograph for analysis. Although the extraction efficiencies of acetaminophen were 0.12–0.13%, the calibration curve showed good linearity in the range of 2.5–200 μg/ml; intra- and inter-day assay coefficients of variation were 5.8 and 13.6% for 100 μg/ml acetaminophen, respectively. The detection limit was about 0.5 μg/ml. Acetaminophen could be actually measured by this method for plasma specimens of volunteers, who had ingested the drug. The above results show that the present SPME method is recommendable for analysis of acetaminophen in forensic toxicology and clinical pharmacology, because of its simplicity and reliability.

Key words: Solid-phase microextraction (SPME); Acetaminophen; 2-Acetamidophenol; Gas chromatography; Flame thermionic detection

Introduction

Acetaminophen (paracetamol) is being widely used as an over-the-counter analgesic-antiinflammatory agent, because of its safety as compared with that of aspirin [1,2]. However, its acute overdosage causes dose-dependent hepatic damages in laboratory animals and humans [3-7]. The damage of the liver caused by the drug is thought due to its reactive metabolites, such as *N*-acetyl-*p*-benzoquinoneimine, which can be covalently bound to cellular macromolecules and thus cause toxicity [8,9]. Acetaminophen hepatotoxicity may be enhanced in chronic alcoholic patients [10,11]. In general, a single acute dose of acetaminophen, at 150 to 250 mg/kg in adults, can result in severe liver damage [12]; its toxic blood concentrations are about 200 μg/ml [2,13]. Its plasma concentration at 4 h after ingestion is useful to predict hepatic damages of patients [14]. Thus it is essential to include acetaminophen in the list of drugs and poisons to be analyzed for emergency drug screening.

*To whom all correspondence should be addressed.
 0915-9606/04/$14.00

Solid-phase microextraction (SPME), a new and simple extraction method, was first introduced by Arthur and Pawliszyn in 1990 [15]. Our group has applied the method to analyses of many drugs and poisons by gas chromatography (GC)[16-22] and high-performance liquid chromatography (HPLC)[23,24]. In this report, we present a simple method for extraction of acetaminophen from plasma by SPME prior to its GC analysis.

Experimental

Materials

Acetaminophen and 2-acetamidophenol were purchased from Sigma (St. Louis,MO, USA) and Aldrich (Milwaukee, WI, USA), respectively. An SPME device, SPME fibers [100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/ divinylbenzene (PDMS/DVB), 75 μm Carboxen/ polydimethylsiloxane (CAR/PDMS), 65 μm Carbowax/ divinylbenzene (CW/DVB) and 85 μm polyacrylate coated fibers] and an SPB-1 fused silica capillary column (15 m x 0.32 mm ID, film thickness 0.25 μm) were purchased from Supelco Inc. (Bellefonte, PA, USA). Pyrinazin® (acetaminophen) was obtained from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). Other common chemicals used were of the analytical grade. Plasma samples were obtained from healthy volunteers.

Extraction procedure

To 1.5 ml of plasma, containing 3.75 to 300 μg acetaminophen and 18.6 μg 2-acetamidophenol, 460 μl of 10% (w/v) zinc sulfate solution was added for deproteinization; the tube was vortex-mixed for 2 min at 80℃ and then centrifuged at 5,000 rpm for 15 min at 4℃. The supernatant fraction was decanted into a 4-ml vial with a small Teflon-coated stirring bar, followed by addition of 0.5 g Na_2SO_4 and 0.3 g $NaHCO_3$. The vial was sealed with a silicone septum cap and placed on an aluminum block heater with a stirrer. After 5 min, the needle of the SPME fiber holder was passed through the septum. Then the fiber in the needle was pushed out and exposed to the sample solution of the vial at 65℃ for 30 min. The fiber was pulled back into the needle; the needle containing the fiber was then quickly pulled out of the septum, and inserted into the GC port to expose the fiber in the injection chamber of GC. The fiber was held there for 5 min before being pulled out of the chromatograph.

GC conditions

GC analysis was performed on a Shimadzu (Kyoto) GC-14B instrument equipped with a flame thermionic detector (FTD). The column temperature was held at 75℃ for 1 min after injection and then programmed up to 280°C at 10℃/min. Injector and detector temperatures were 200 and 270℃, respectively, and the helium flow rate was about 3.0 ml/min. The injection was set in the splitless mode, and the splitter was opened 1 min after the insertion of the SPME fiber.

Administration of acetaminophen to humans

Five hundred milligrams of acetaminophen (Pyrinazin®) was orally administered to two healthy male volunteers (41 and 45 years old). One and two hours after the administration, 7 ml each of blood was sampled. The plasma samples were stored at –80°C, and analyzed on the next day.

Results

We tested various types of SPME fibers, such as polyacrylate, PDMS, PDMS/DVB, CAR/PDMS and CW/DVB. The extraction efficiency for acetaminophen was highest with polyacrylate, followed by CW/DVB; the efficiencies with other fibers were less than 17 % of that of the polyacrylate-coated fiber (Fig. 1). We thus adopted the polyacrylate fiber in this study. We also tried some salts to obtain higher efficiencies; Na_2SO_4 plus $NaHCO_3$ gave higher efficiency than ammonium citrate or NaCl. The optimum amounts of Na_2SO_4 and $NaHCO_3$ were 0.5 and 0.3 g, respectively in a vial. We tested various temperatures for SPME, such as 40, 50, 55, 60, 65, 70 and 75°C, and got the best result at 65°C (data not shown). These optimal conditions were adopted in the present SPME procedure.

Figure 2 shows typical gas chromatograms of acetaminophen and IS. The top panel shows the chromatogram for the authentic acetaminophen and IS dissolved in methanol (100 and 50 ng on-column, respectively), and the bottom panel shows that for the SPME extract obtained from human plasma containing spiked acetaminophen (100 μg/ml) and IS (12.5 μg/ml). The retention

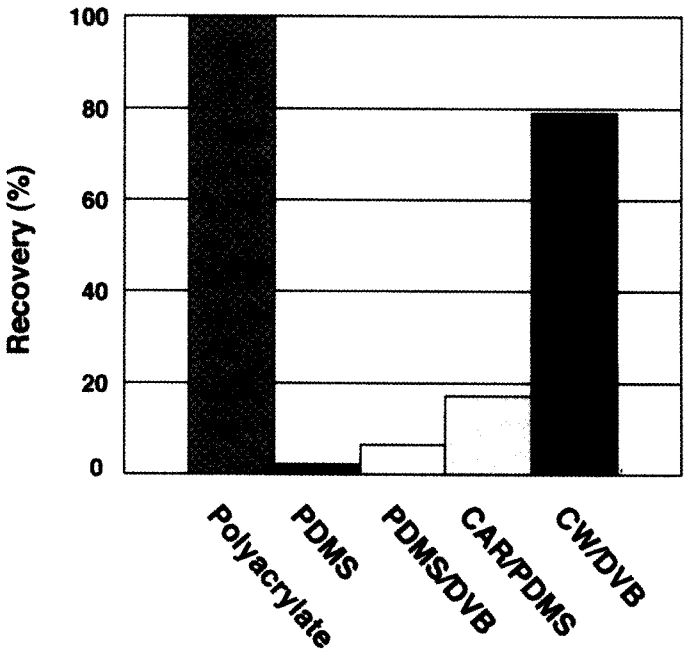

Fig. 1. Comparison of five SPME fiber coatings, *i. e.* , polyacrylate, polydimethylsiloxane (PDMS), polydimethylsiloxane/ divinylbenzene (PDMS/DVB), Carboxen/polydimethylsiloxane (CAR/PDMS) and Carbowax/divinylbenzene (CW/DVB) fibers for extraction of acetaminophen from plasma (50 μg/ml). Each SPME fiber was exposed to the sample solution at 65℃ for 30 min. The amount of acetaminophen extracted by the polyacrylate fiber was set at 100%.

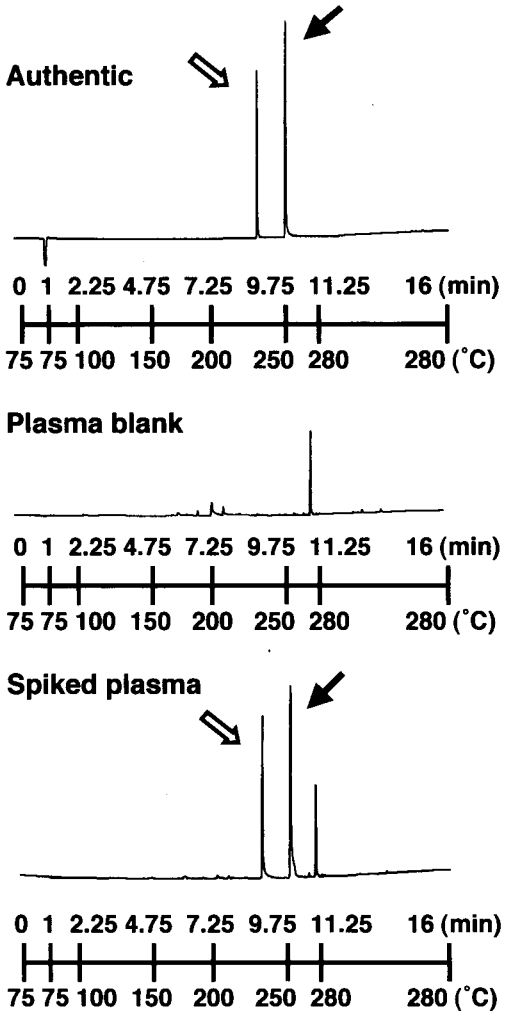

Fig. 2. Gas chromatograms for 100 ng each of the non-extracted authentic acetaminophen (filled arrow) and IS (open arrow) dissolved in methanol with direct injection (top panel), for the SPME extract from human plasma spiked with 100 μg/ml of acetaminophen and 12.5 μg/ml of IS (bottom panel) and for the extract from blank plasma (middle panel).

times were 9.0 min for acetaminophen and 10.0 min for IS. No interfering peaks were found as shown in blank plasma (middle panel).

The extraction efficiencies for acetaminophen were calculated by comparing the peak areas for the SPME extracts with that for the authentic acetaminophen dissolved in methanol; they were 0.13 and 0.12% for 20 and 100 μg/ml acetaminophen in plasma, respectively.

The calibration curve was drawn by plotting seven different points in the range of 2.5-200 μg/ml. It gave good linearity, and the equation was $y = 0.0148\,x + 0.191$ with the r value of 0.996. The detection limit of the drug was about 0.5 μg/ml.

The coefficients of variation (CV) for intra-day measurements (n=4) were 15.6% for 20 μg/ml acetaminophen and 5.8% for 100 μg/ml acetaminophen; the inter-day CVs (n=5) were 21.0% for 20 μg/ml and 13.6% for 100 μg/ml.

Two healthy volunteers were orally administered 0.5 g of acetaminophen (Pyrinazin®); their blood specimens were sampled one and two hours after administration to be analyzed by the present SPME method. Figure 3 shows gas chromatograms obtained from plasma of one of the subjects. The results of the levels of the drug in plasma of the two subjects are shown in Table 1.

Discussion

In this report, we have presented a simple method for extraction of acetaminophen in human plasma using SPME. Acetaminophen was determined or identified by GC-flame ionization detector (FID)[25], GC-electron capture detector (ECD)[26], GC/mass spectrometry (MS)[27,28], HPLC [29-41] and HPLC/MS [42].

Its limits of detection or quantitation were about 7.6 μg/ml by GC-FID [25], 1 pg on-column by GC/MS [27], 0.025–5 μg/ml

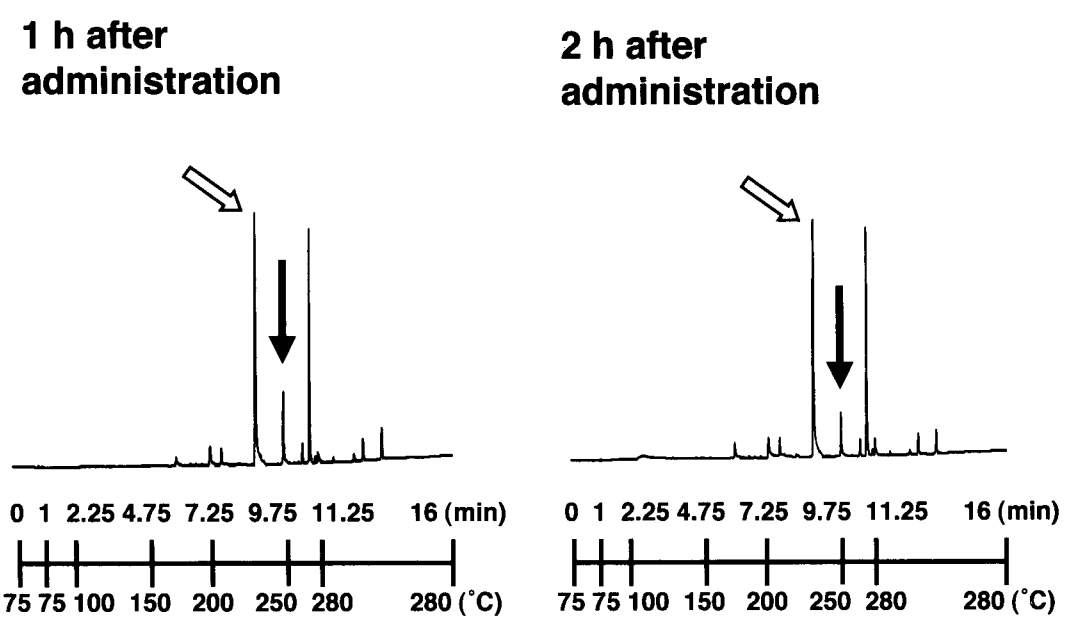

Fig. 3. Gas chromatograms for SPME extracts of plasma samples of a volunteer, who took 0.5 g of acetaminophen (Pyrinazin®); the plasma samples were obtained one and two hours after its oral administration. In both panels, filled and open arrows show the peaks of acetaminophen and IS, respectively.

Table 1. Acetaminophen concentrations in serum samples of volunteers after its oral administration (0.5 g)

Subject	Time after administration (h)	Acetaminophen concentration (μg/ml)
1	1	18.7
	2	11.5
2	1	11.5
	2	7.80

by HPLC [31,32-35,37,39-41]. In some methods, appropriate derivatization was necessary for its detection [26-28]. The detection limit obtained by our method (0.5 μg/ml) is much lower than those by GC-FID.

Therapeutic concentrations of acetaminophen is 10–25 μg/ml [3]; thus the present method is sufficiently applicable to the measurements of the drug at therapeutic and toxic levels.

In conclusion, our method is recommendable for the detection of acetaminophen in clinical and toxic cases, because of its simplicity, precision and sufficient sensitivity.

References

1) Roberts II LJ, Morrow JD: Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In Hardman JG, Limbird LE, Goodman Gilman A (eds), The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, New York, 2001, pp. 687-731.

2) Moffat AC, Jackson JV, Moss MS, Widdop B (eds): Clarke's Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986, pp. 849-850.

3) Prescott LF, Roscoe P, Wright N, Brown SS: Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. Lancet, **i**, 519-522 (1971).

4) Prescott LF, Wright N: The effects of hepatic and renal damage on paracetamol metabolism and excretion following overdosage. A pharmacokinetic study. Br J Pharmacol, **49**, 602-613 (1973).

5) Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB: Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmacol Exp Ther, **187**, 185-194 (1973).

6) Moldéus P: Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. Biochem Pharmacol, **27**, 2859-2863 (1978).

7) Poulsen HE, Lerche A, Skovgaard LT: Acetaminophen metabolism by the perfused rat liver twelve hours after acetaminophen overdose. Biochem Pharmacol, **34**, 3729-3733 (1985).

8) Corcoran GB, Mitchell JR, Vaishnav YN, Horning EC: Evidence that acetaminophen and *N*-hydroxyacetaminophen form a common arylating intermediate, *N*-acetyl-*p*-benzoquinoneimine. Mol Pharmacol, **18**, 536-542 (1980).

9) Dahlin DC, Miwa GT, Lu AYH, Nelson SD: *N*-Acetyl-*p*-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA, **81**, 1327-1331 (1984).

10) Slattery JT, Nelson SD, Thummel KE: The complex interaction between ethanol and acetaminophen. Clin Pharmacol Ther, **60**, 241-246 (1996).

11) Prescott LF: Paracetamol, alcohol and the liver. Br J Clin Pharmacol, **49**, 291-301 (2000).

12) Prescott LF: Paracetamol overdosage. Pharmacological considerations and clinical management. Drugs, **25**, 290-314 (1983).

13) Stead AH, Moffat AC: A collection of therapeutic, toxic and fatal blood drug concentrations in man. Human Toxicol, **3**, 437-464 (1983).

14) Rumack BH, Peterson RC, Koch GG, Amara IA: Acetaminophen overdose. Arch Intern Med, **141**, 380-385 (1981).

15) Arthur CL, Pawliszyn J: Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem, **62**, 2145-2148 (1990).

16) Kumazawa T, Lee X-P, Tsai M-C, Seno H, Ishii A, Sato K: Simple extraction of tricyclic antidepressants in human urine by headspace solid-phase microextraction (SPME). Jpn J Forensic Toxicol, **13**, 25-30 (1995).

17) Kumazawa T, Watanabe K, Sato K, Seno H, Ishii A, Suzuki O: Detection of cocaine in human urine by solid-phase microextraction and capillary gas chromatography with nitrogen-phosphorus detection. Jpn J Forensic Toxicol, **13**, 207-210 (1995).

18) Seno H, Kumazawa T, Ishii A, Nishikawa M, Watanabe K, Hattori H, Suzuki O: Detection of some phenothiazines by headspace solid phase microextraction (SPME) and gas chromatography. Jpn J Forensic Toxicol, **14**, 30-34 (1996).

19) Ishii A, Seno H, Kumazawa T, Nishikawa M, Watanabe K, Hattori H, Suzuki O: Simple clean-up of methamphetamine and amphetamine in human urine by direct-immersion solid phase micro extraction (DI-SPME). Jpn J Forensic Toxicol, **14**, 228-232 (1996).

20) Guan F, Watanabe K, Ishii A, Seno H, Kumazawa T, Hattori H, Suzuki O: Headspace solid-phase microextraction and gas-chromatographic determination of dinitroaniline herbicides in human blood, urine and environmental water. J Chromatogr B, **714**, 205-213 (1998).

21) Guan F, Seno H, Ishii A, Watanabe K, Kumazawa T, Hattori H, Suzuki O: Solid-phase microextraction (SPME) and GC-ECD of benzophenones for detection of benzodiazepines in urine. J Anal Toxicol, **23**, 54-61 (1999).

22) Kumazawa T, Seno H, Lee X-P, Ishii A, Watanabe-Suzuki K, Sato K, Suzuki O: Extraction of methylxanthines from human body fluids by solid-phase microextraction. Anal Chim Acta, **387**, 53-60 (1999).

23) Kurihara R, Ishii A, Watanabe-Suzuki K, Kumazawa T,

Suzuki O, Seno H, Katsumata Y: Simple extraction of gamma-hydroxybutyrate in human whole blood by headspace solid-phase microextraction. Jpn J Forensic Toxicol, **19**, 38-45 (2001).
24) Kumazawa T, Seno H, Watanabe-Suzuki K, Hattori H, Ishii A, Sato K, Suzuki O: Determination of phenothiazines in human body fluids by solid-phase microextraction and liquid chromatography/tandem mass spectrometry. J Mass Spectrom, **35**, 1091-1099 (2000).
25) Kaa E: Rapid gas chromatographic method for emergency determination of paracetamol in human serum. J Chromatogr, **221**, 414-418 (1980).
26) Chan K and McCann JF: Improved gas-liquid chromatography-electron-capture detection technique for the determination of paracetamol in human plasma and urine. J Chromatogr, **164**, 394-398 (1979).
27) Murray S, Boobis AR: Combined assay for phenacetin and paracetamol in plasma using capillary column gas chromatography-negative-ion mass spectrometry. J Chromatogr, **568**, 341-350 (1991).
28) Speed DJ, Dickson SJ, Cairns ER, Kim ND: Analysis of paracetamol using solid-phase extraction, deuterated internal standards, and gas chromatography-mass spectrometry. J Anal Toxicol, **25**, 198-202 (2001).
29) Buskin JN, Upton RA, Williams RL: Improved acetaminophen assay sensitivity by modification of a high-performance liquid chromatography technique. J Chromatogr, **230**, 443-447 (1982).
30) Wilson JM, Slattery JT, Forte AJ, Nelson SD: Analysis of acetaminophen metabolites in urine by high-performance liquid chromatography with UV and amperometric detection. J Chromatogr, **227**, 453-462 (1982).
31) Jung D, Ul Zafar N: Micro high-performance liquid chromatographic assay of acetaminophen and its major metabolites in plasma and urine. J Chromatogr, **339**, 198-202 (1985).
32) Colin P, Sirois G, Chakrabarti S: Rapid high-performance liquid chromatographic assay of acetaminophen in serum and tissue homogenates. J Chromatogr, **413**, 151-160 (1987).
33) Ladds G, Wilson K, Burnett D: Automated liquid chromatographic method for the determination of paracetamol and six metabolites in human urine. J Chromatogr, **414**, 355-364 (1987).
34) Kinney CD, Kelly JG: Liquid chromatographic determination of paracetamol and dextropropoxyphene in plasma. J Chromatogr, **419**, 433-437 (1987).
35) Bhargava VO, Emodi S, Hirate J: Quantitation of acetaminophen and its metabolites in rat plasma after a toxic dose. J Chromatogr, **426**, 212-215 (1988).
36) Aguilar MI, Hart SJ, Calder IC: Complete separation of urinary metabolites of paracetamol and substituted paracetamols by reversed-phase ion-pair high-performance liquid chromatography. J Chromatogr, **426**, 315-333 (1988).
37) Mathis DF, Budd RD: Extraction of acetaminophen and theophylline from post-mortem tissues and urine for high-performance liquid chromatographic analysis. J Chromatogr, **439**, 466-469 (1988).
38) Osterloh J, Yu S: Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens. Clin Chim Acta, **175**, 239-248 (1988).
39) Kamali F, Herd B: Liquid-liquid extraction and analysis of paracetamol (acetaminophen) and its major metabolites in biological fluids by reversed-phase ion-pair chromatography. J Chromatogr, **530**, 222-225 (1990).
40) Brunner LJ, Bai S: Simple and rapid assay for acetaminophen and conjugated metabolites in low-volume serum samples. J Chromatogr B, **732**, 323-329 (1999).
41) Campanero MA, Calahorra B, García-Quétglas E, López-Ocáriz A, Honorato J: Rapid liquid chromatographic assay for the determination of acetaminophen in plasma after propacetamol administration: application to pharmacokinetic studies. J Pharm Biomed Anal, **20**, 327-334 (1999).
42) Betowski LD, Kofmacher WA, Lay Jr JO, Potter DW, Hinson JA: Direct analysis of rat bile for acetaminophen and two of its conjugated metabolites via thermospray liquid chromatography/mass spectrometry. Biomed Environ Mass Spectrom, **14**, 705-709 (1987).