Isomeric Separation of Methamphetamine by HPLC Chiral Column

VEERAVAN LEKSKULCHAI, Ph.D.*

Abstract

Methamphetamine and its active metabolite, amphetamine, are optically active compounds which, based upon synthetic routes, can be found in two forms; pure d-form and racemic mixture. Analysis of their isomers can help to identify which precursor is currently spreading widely in a given region. Since there are many drugs that can be metabolized to amphetamine/methamphetamine, isomeric separation can be a useful tool for evaluation of these drugs, as well. Indirect method by using N-trifluoroacetyl- l -prolyl chloride (l -TPC) was found to have limited accuracy due to the contribution effect. In this presentation a direct method using HPLC Chirex chiral column 3022 was studied. Although the method gave no base-line separation of two different isomer peaks, it gave good sensitivity, reliability, and linearity. No contribution effect was found in the method presented. It also gave excellent correlation with the l-TPC method.

Key word : Methamphetamine, Isomeric Separation, Chiral Column

LEKSKULCHAI V
J Med Assoc Thai 2001; 84: 1604-1613

Methamphetamine has a chiral or asymmetric carbon in its molecular structure, thus, it can be found in two different optical isomers; dextrorotatory (d- or (+)-isomer) and levorotatory (l- or (-)- isomer) (Fig. 1). Depending on the synthetic reaction, street methamphetamine can be found in two different forms; the pure d-form and the equal mixture of d- and l -isomers so called "racemic mixture"(1-3). Methamphetamine was first synthesized from phenyl-2-propanone (P-2-P); a simple compound, the product from this precursor is in a racemic mixture. After P-2-P was classified as a controlled substance in 1981, the precursor for methamphetamine synthesis has been changed to ephedrine

* Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10300, Thailand.

(4). The new method gives a pure d-methamphetamine(1). A few years ago, ephedrine was also classified as a controlled substance (Controlled substance act 1997), leading to a question of coming back of the cheaper precursor, P-2-P, in the street market. Therefore, isomeric separation of methamphetamine can help to find out which method is presently used in the market. This information is necessary for law enforcement to effectively control methamphetamine synthesis(1,5,6). d- and l -isomers also have differences in various potencies. d-Isomer is more potent to CNS stimulation, it has; thus, more potential for abuse. Since the l -form can cause several unpleasant side-effects such as stomach cramping and a pounding heart, it can probably stop abusers from taking overdoses(7-9). It is dangerous for abusers who use drugs without medical supervision and do not know the actual dose and isomeric form of methamphetamine being taken. The isomeric separation of methamphetamine can be used as a tool to identify which form is currently spreading in a specific region. In addition, there are medications such as benzphetamine(10), famprofazone(11), selegiline(12) that are metabolized to methamphetamine and/or amphetamine as well. Isomeric separation is helpful in drug development in order to determine whether these drugs are metabolized to d-isomer which could induce drug addiction. In this presentation, a method for separating isomers of methamphetamine was developed and compared with the GC/MS method previously available(13).

MATERIAL AND METHOD

All reagents used in this study are chromatographic grade purchased from Fisher Scientific Products (Fair Lawn, NJ). N-methylphenethylamine and benzoyl chloride were purchased from Sigma Co. (St. Louis, MO). N-trifluoroacetyl- l -prolyl chloride (l -TPC) was obtained from Regis Chemical Company (Grove, IL). All stock standards (1 mg/mL) were purchased from Radian Co. (Austin, TX). The HPLC chiral column is the 250 x 4.6 mm Chirex chiral stationary phase 3022 purchased from Phenomenex (Torrance, CA). The column is a bush type of d-indole carboxylic acid and α- l-naphthylamine. High performance liquid chromatography (HPLC) analysis was performed on a Hewlett Packard 1090 with diode array detector. Gas chromatography/mass spectrometry (GC/MS) was performed on the Varian Star 3400Cx (Sugar Land, TX) equipped with a 30 m x 0.25 mm DB5 capillary column (coated with 5% phenyl polysiloxane) purchased from J&W Scientific (Folsum, CA).

Drug free urine samples were obtained from healthy volunteers. They were pooled and kept in the refrigerator. The pooled urine was used to prepare samples with known amounts of specific drugs. Two mL of urine was alkalinized and then extracted

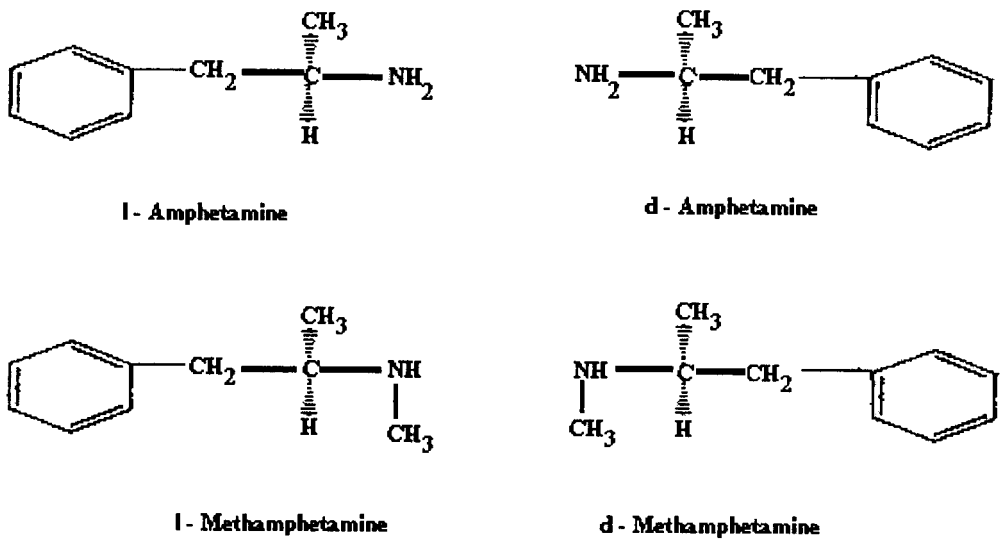


Fig. 1. Molecular structures of amphetamine and methamphetamine isomers.

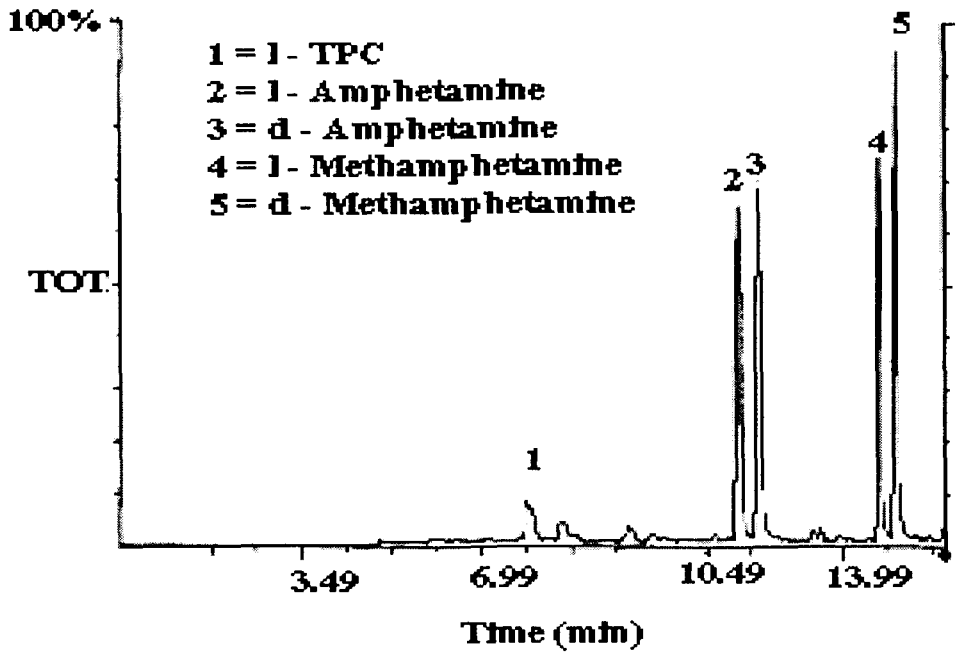


Fig. 2. Gas chromatogram of l-TPC-derivatives.

with 2 mL of hexane. The hexane layer was derivatized with l-TPC for GC method or benzoyl chloride for HPLC method. The extract was evaporated to dryness, reconstituted in 50 µL of hexane, and then 2 µL was injected into the analyzers. The mobile phase used in this study was a mixture of hexane and isopropanol (90 : 10).

RESULTS

The indirect method using l-TPC derivatization and separating on GC/MS was set as a reference method. The GC chromatogram is shown in Fig. 2, Fig. 3 shows the GC chromatograms of two urine samples; one was spiked with d-amphetamine and the other was spiked with l -methamphetamine. As expected, these chromatograms show the contribution effect of l -TPC derivatization. The new method using HPLC chiral column gave a chromatogram as shown in Fig. 4. N-methylphenethylamine was used as the internal standard. No contribution effect was found when urine spiked with d-methamphetamine was analyzed with this HPLC method (Fig. 5). By using the peak height ratio, the limit of detection of the HPLC method was at the concentration of 250 ng/mL and the linearity was up to 5,000 ng/mL (Fig. 6). The percentages of coefficient of variance representing within run precision of the HPLC method are shown in Table 1. The correlation between the GC method and the HPLC method was studied and the results are shown in Fig. 7.

DISCUSSION

Amphetamine and methamphetamine are optically active compounds. Most of the immunoassays as well as chromatographic confirmation tests for these analytes do not differentiate their d- and l-isomers. Isomers of amphetamine and methamphetamine were first analyzed using the traditional approach of derivatization with l-TPC, a chiral derivatizing reagent (CDR), followed by the separation on a routinely used non-chiral chromatographic columns. A critically important question in using the CDR is its enantiomeric purity. The presence of an optically active contaminant; in this case is the contamination of d-TPC, will result in a false value for the enantiomeric composition of drug isomers[14]. This phenomenon is called "contribution effect". When two chiral compounds, race-

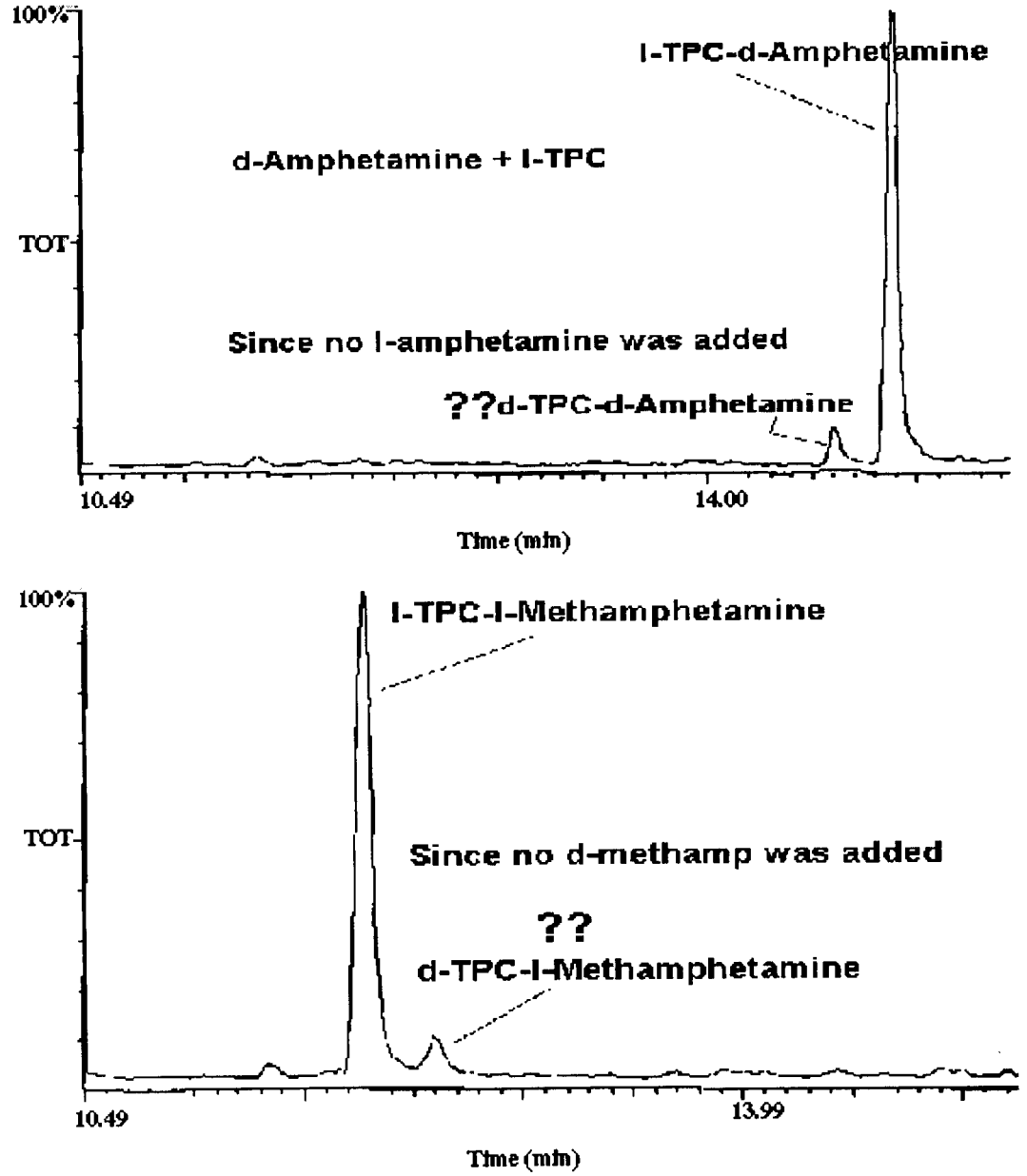


Fig. 3. Contribution effect found in l-TPC method.

mic A (representing drug isomers needed to be analyzed) and racemic B (representing the CDR and its optically active contaminant), react to form covalent adducts in a reaction that does not affect the asymmetric centers, the stereochemical course of the reaction may be depicted as follows:

$$(\pm)\text{-A} + (\pm)\text{-B} \text{ ----> } \underset{1}{(+A+B)} + \underset{2}{(+A-B)} + \underset{3}{(-A+B)} + \underset{4}{(-A-B)}$$

where (+) = d-, (-) = l-, and (±) = racemic mixture

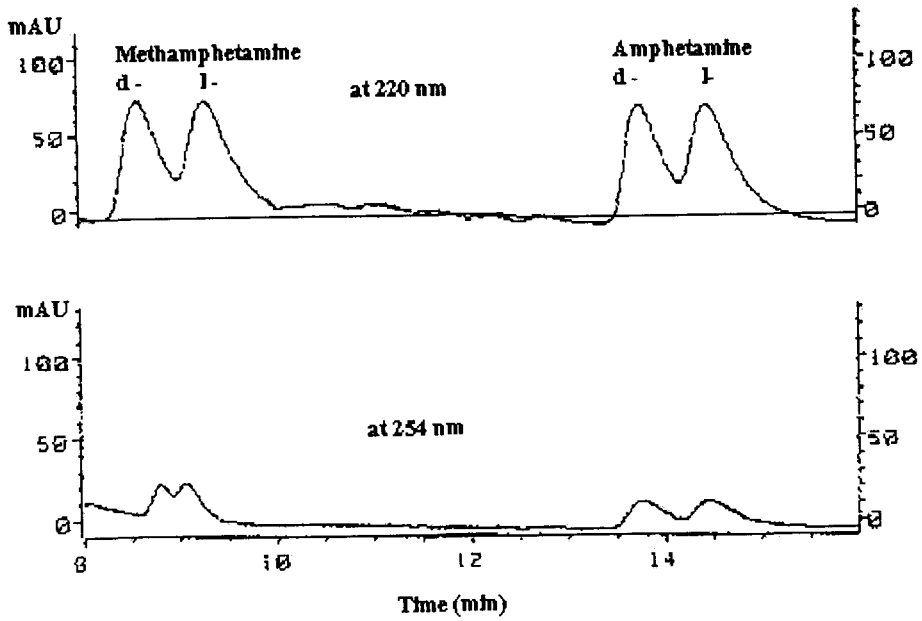


Fig. 4. HPLC chromatogram of amphetamine and methamphetamine isomers on chiral column.

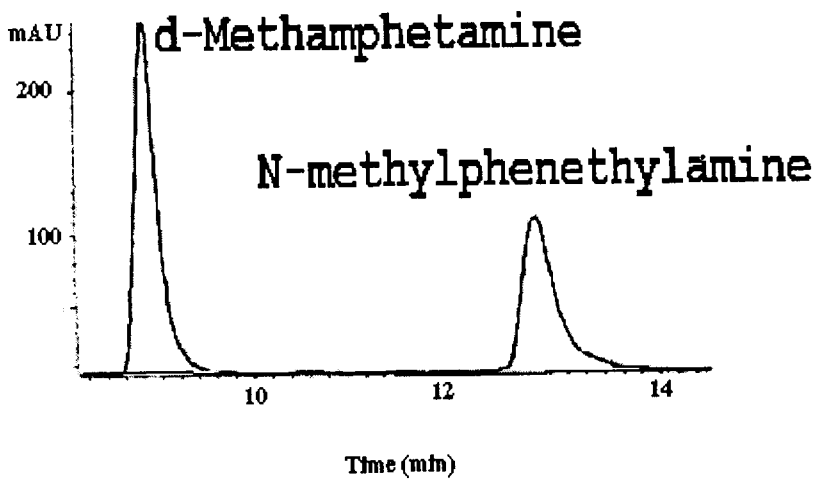
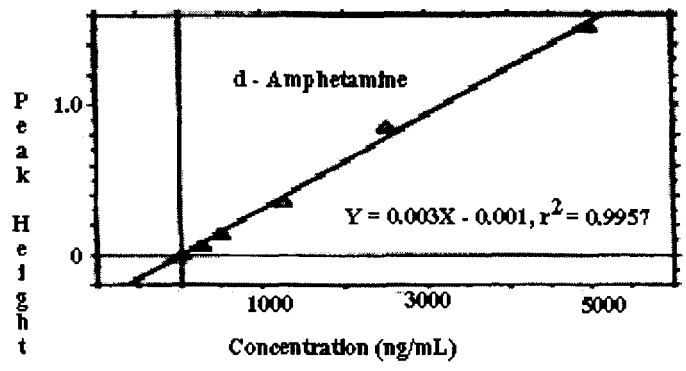
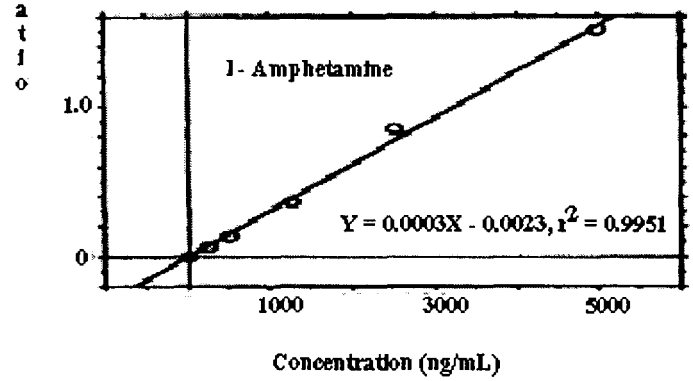
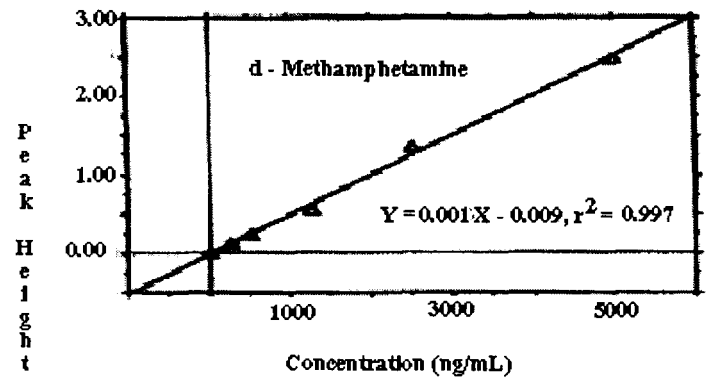


Fig. 5. HPLC chromatogram of d-methamphetamine showing no contribution effect.

Derivatives 1 and 4 are enantiomers of each other, as are 2 and 3. Derivatives 1 and 3 as well as 2 and 4 are diastereomeric pairs. Non-chiral chromatographic systems separate a diastereomeric pair, but not enantiomers. Thus, chromatographic analysis of a mixture of these four products in a non-chiral chromatographic system can yield two peaks (peak of 2 and 3 and peak of 1 and 4)[15].







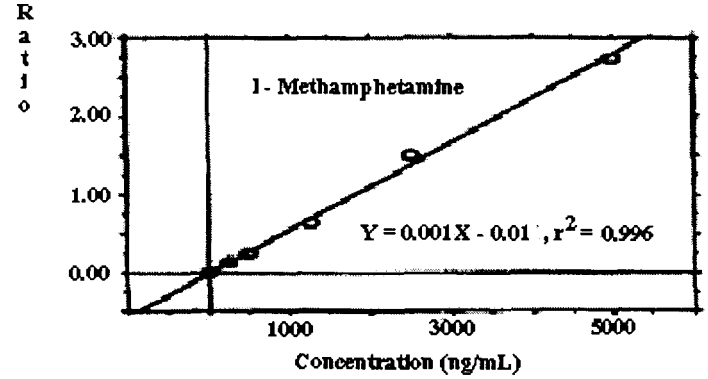


Fig. 6. Standard curves of d- and l-amphetamine (Amp) and d- and l-methamphetamine (Meth) by HPLC method.

Table 1. Urine HPLC amphetamine and methamphetamine enantiomeric analyses: within-run variations.

Added concentration (ng/mL)	Mean	SD	% CV
d-Amphetamine			
500 ng/mL	461	28	6
2,500 ng/mL	2,584	52	2
l-Amphetamine			
500 ng/mL	479	38	8
2,500 ng/mL	2,537	127	5
d-Methamphetamine			
500 ng/mL	468	19	4
2,500 ng/mL	2,259	68	3
l-Methamphetamine			
500 ng/mL	473	28	6
2,500 ng/mL	2,287	69	3

Note: n = 5 at each concentration

From the diagram above, contamination of d-TPC will lead to a formation of derivatives d-TPC-d-amphetamine (1) and d-TPC-l-amphetamine (3) which co-elute with derivatives l-TPC-l-amphetamine (4) and l-TPC-d-amphetamine (2), respectively. In this presentation, the contribution effect shown in Fig. 3 was found even when the l-TPC was first opened. The effect was inconstantly increased when the aged l-TPC was used despite being kept in the refrigerator. Arguments against the use of CDRs include: (1) the length of time involved for the derivatization reaction which depends on the kinetic reaction rate of each isomer, (2) the possibility of racemization of CDRs during the formation of diastereomers or storage, (3) the variability in the formation rates of diastereomers which may cause a kinetic resolution during the separation[16]. Furthermore, the reactions of each isomer with CDR (reaction between 2 enantiomeric compounds) have different kinetic reaction rates. In order to make sure all isomers get complete derivatization, a long derivatization time is necessary[17]. Direct chiral separation methods using a chiral stationary phase (CSP) have been developed in order to eliminate these disadvantages of indirect methods and to enhance the accuracy of chiral analysis.

Theoretically, no derivatization is necessary for direct HPLC chiral separation. However, most amines including amphetamine and methamphetamine have very poor UV absorption (extinction coefficient of amphetamine and methamphetamine in aqueous acid solution at the wavelength of 257 nm are 14 and 12 L/g/cm, respectively). Only by chemical derivatization can therapeutic and subtherapeutic amounts of amines be detected by HPLC [18]. From this presentation, the UV absorption of amphetamine and methamphetamine was lower when they were put in the mobile phase (calculated extinction coefficients of both amphetamine and methamphetamine in hexane at the wavelength of 200 nm are 6.2 L/g/cm). Although derivatization requires additional effort, the reagents used are typically simple, inexpensive, easily used, and are chosen to facilitate detection, separation, and quantitation. Since achiral derivatization reagents react with both enantiomers in an identical fashion and at identical rates, the ratio of the derivatized analyte is always the same as that of the enantiomeric precursors. It is not necessary that the reaction proceed to completion. Moreover, detector response is the same for achiral derivatives of both enantiomers. This is not necessarily true for diastereomeric derivatives[19].

Nagai and Kamiyama[20] successfully applied Chiralcel OB and OJ columns (type 2 CSP that allows solutes to enter into the chiral cavities forming inclusion complexes) for the enantiomer separation of benzoyl derivatives of methamphetamine and amphetamine. The CSP used in this study is type 1 CSP that was chosen for rapid analysis. The analytes interact with the specific groups on the surface of the CSP. The presented data showed that

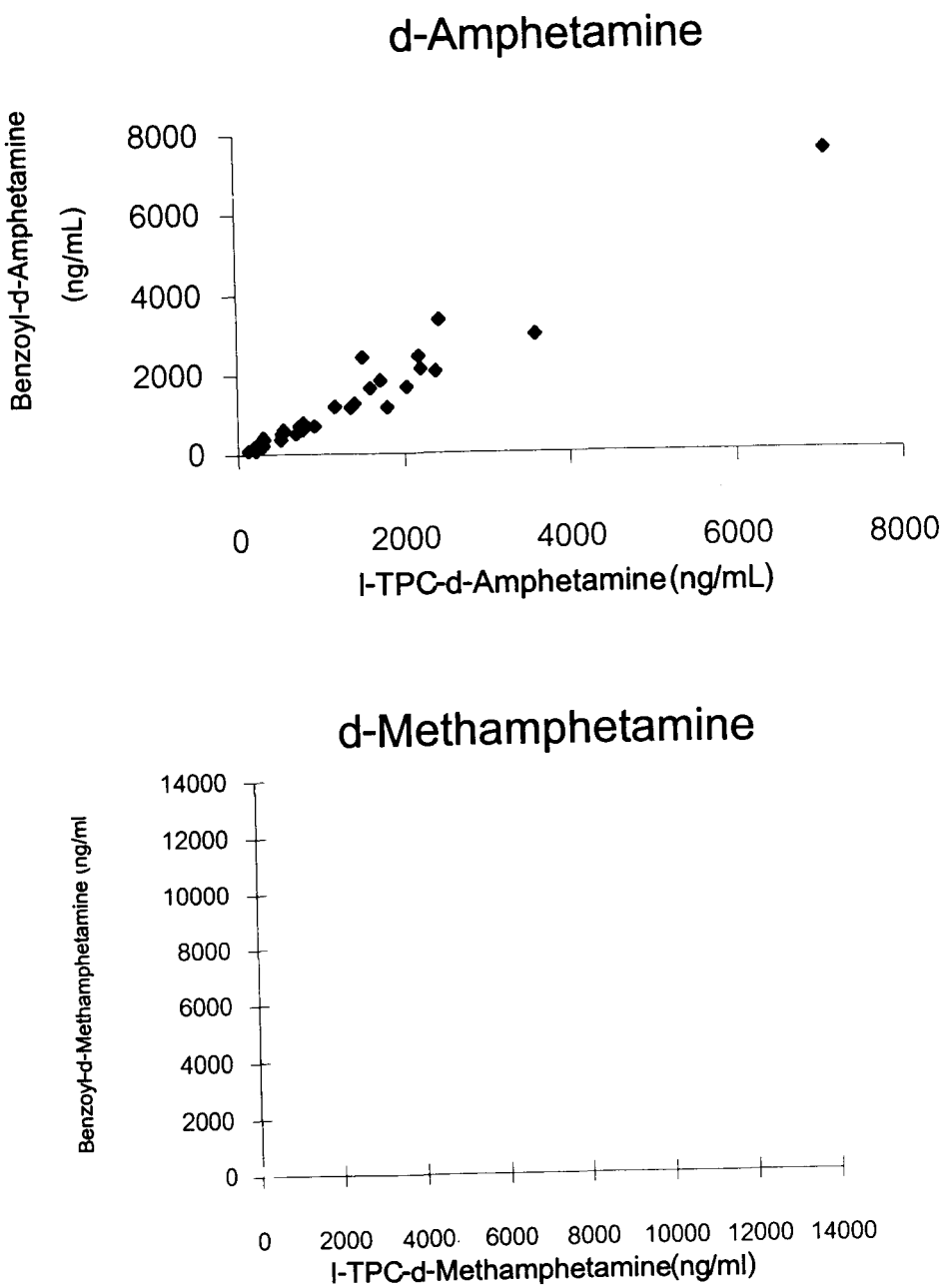


Fig. 7. Correlation between l-TPC method and HPLC method for d-amphetamine (N = 100, Y = 1.07X - 91.78 and r^2 = 0.97) and for d-methamphetamine (N = 100, Y = 0.96X + 194.29 and r^2 = 0.94).

no baseline separation was found from any enantiomeric separation of benzoyl derivatives. This may in part be due to the weak interaction between analytes and CSP.

Although the benzoyl derivatives did not yield baseline separation, by using peak height for quantitation, acceptable linearity, precision and sensitivity were obtained. The linearity of benzoyl

methamphetamine isomers is up to 5,000 ng/ml. Compared with l-TPC method, benzoyl chloride derivatization required shorter reaction time (20 min *vs* 1 h). Nonetheless, both methods need an equal detection time (16 min) and a good correlation was observed when comparing the methods.

(Received for publication on April 23, 2001)

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การตรวจแยกไอโซเมอร์ของเม็ธแอมเฟตามีนด้วย HPLC Chiral Column

*วีรวรรณ เล็กสกุลไชย, ปร.ด.**

ยาบ้า หรือเม็ธแอมเฟตามีน และแอมเฟตามีนเป็นสารที่มีไอโซเมอร์พบได้สองรูปแบบคือรูปที่เป็น d-form และที่เป็นรูปผสมของ d- และ l-forms ที่เรียกว่า racemic mixture ขึ้นกับวิธีการผลิต การตรวจแยกชนิดไอโซเมอร์ของ ยาบ้าจะช่วยบอกว่าวิธีการผลิตยาบ้าที่ใช้อยู่ในแต่ละท้องที่คืออะไร นอกจากนี้มียาจำนวนมากที่เมื่อรับเข้าสู่ร่างกายสามารถถูกเปลี่ยนเป็นยาบ้า ทำให้การตรวจแยกชนิดไอโซเมอร์ของยาบ้ามีบทบาทในการประเมินผลเสียจากการใช้ยาเหล่านี้ วิธีการที่มีใช้อยู่เดิมในการตรวจแยกชนิดของยาบ้าจะใช้การทำปฏิกิริยากับ l-TPC วิธีการนี้มีผลเสียคือให้ผลการตรวจที่ไม่เที่ยงตรงจาก contribution effect หลักการที่นำเสนอใช้ HPLC Chirex chiral column 3022 ในการตรวจแยกไอโซเมอร์ของยาบ้า แม้ว่าหลักการนี้ให้ผลการแยกของ peak ไม่สมบูรณ์ แต่สามารถให้ความไว ความถูกต้องและความเป็นเส้นตรงที่ดี รวมทั้งไม่มีปัญหาเรื่อง contribution effect เมื่อศึกษาเปรียบเทียบกับหลักการเดิมที่ใช้ l-TPC พบว่าให้ผลการตรวจวิเคราะห์ไปในทิศทางเดียวกันและใกล้เคียงกัน

คำสำคัญ : ยาบ้า, การตรวจแยกไอโซเมอร์, ชิราล คอลั่มน์

วีรวรรณ เล็กสกุลไชย
จดหมายเหตุทางแพทย์ ฯ 2544; 84: 1604-1613

* ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ, กรุงเทพ ฯ 10300