DETERMINATION OF TROPICAMIDE ABUSE IN HAIR WITH A VALIDATED GC-MS METHOD

Zeynep Türkmen[1], Selda Mercan[1], Sıtkı Bağdadi[2], Salih Cengiz[1]
[1]Istanbul University, Institute of Forensic Sciences, Istanbul
[2]Kartal Yavuz Selim State Hospital, Department of Anesthesiology and Reanimation, Istanbul

ABSTRACT

Objective: *Tropicamide is an antimuscarinic drug used topically by clinicians prior to eye examination for its cycloplegic and mydriatic effects. It is included in the controlled drug-list since the second half of 2012 in Turkey due to abuse potential. There is no literature concerning the determination of tropicamide from hair sample. In this study, tropicamide determination method was validated partially by GC-MS and applied on an abuser's hair sample.*

Material and Method: *A validated method for tropicamide determination by GC-MS from hair was developed.*

Results: *Retention time of Tropicamide was found to be 12.79 min, linear range was between 15-1200 ng/mg with 0.998 correlation coeff.; accuracy, recovery and precision values were satisfactory. The method was successfully applied to a tropicamide abuser case and determined 63.61 ng/mg in hair.*

Conclusion: *This is the first report related to tropicamide abuse in hair in the literature. It was concluded that hair analysis is reliable technique to identify past substance abuse and it is necessary to reveal new strategies and notice the new trends of drug abuse.*

Keywords: *Tropicamide, cycloplegics, drug abuse, hair, GC-MS.* ***Nobel Med 2015; 11(3): 50-54***

GEÇERLİ KILINMIŞ BİR GC-MS YÖNTEMİ İLE SAÇTAN TROPİKAMİD İSTİSMARININ BELİRLENMESİ

ÖZET

Amaç: Tropikamid, siklopejik ve midriyatik etkisinden dolayı göz muayenesi öncesi klinisyenler tarafından lokal olarak kullanılan antimuskarinik bir ilaçtır. İstismar potansiyeli dolayısıyla Türkiye'de 2012'nin ikinci yarısından bu yana kontrollü ilaç listesinde yer almaktadır. Tropikamidin saç örneğinden belirlenmesine ilişkin bir literatür bulunmamaktadır. Bu çalışmada, GC-MS ile tropikamid belirleme yöntemi kısmen valide edilmiş ve bağımlının saç örneğinde uygulanmıştır.

Materyal ve Metot: Saçta GC-MS ile tropikamidin belirlenmesi için geçerli bir yöntem geliştirilmiştir.

Bulgular: Tropikamidin alıkonma zamanı 12,79 dk, doğrusal aralığı 15-2000 ng/mg, korelasyon katsayısı 0,998; doğruluk, geri kazanım ve kesinlik değerleri kabul edilebilir bulunmuştur. Tropikamid bağımlısı bir olguya yöntem başarılı bir şekilde uygulanmış ve saçında 63,61 ng/mg belirlenmiştir.

Sonuç: Bu çalışma, saçta tropikamid istismarının belirlenmesi ile ilgili literatürdeki ilk araştırmadır. Saç analizlerinin, uyuşturucu madde kullanım geçmişinin belirlenmesinde güvenilir olduğu ve ilaç istismarında yeni eğilimlerin belirlenmesinde ve yeni stratejilerin ortaya çıkarılmasında gerekli olduğu sonucuna varılmıştır.

Anahtar kelimeler: Tropikamid, sikloplejikler, ilaç istismarı, saç, GC-MS **Nobel Med 2015; 11(3): 50-54**

INTRODUCTION

There are various illegal substances in circulation and route of administration. Besides, new ones are still added into the controlled drug list day by day. It is hard but also necessary to identify and monitor new drugs and methods of consumption in time. Abuse of anticholinergic drugs has been known for a long time. The first case with anticholinergic drug abusing was reported in 1960.[1] Cycloplegic drugs are generally muscarinic receptor blockers including atropine, cyclopentolate, homatropine, scopol-amine and tropicamide (TPC). TPC, the active ingredient contained in ophthalmic eye drops, is an antimuscarinic drug which produces short acting mydriasis (dilation of the pupil) and cycloplegia and abused because of its stimulating effects, which called deliriant.[2] It has a shorter duration of action than the other cycloplegic drugs such as cyclopentolate hence it is more effective for systemic actions.[3-5] On the other hand, it has antiparkinsonian effects due to the modest M4 selectivity when compared to those of atropine on motor vs cognitive tasks.[6] It is used during eye examination to better visualize the retina.[7,8] It is also very popular among teenagers, especially in Russia.[9] It has been included in the controlled drug-list since the second half of 2012 in Turkey, due to abuse potential.

In an overdose situation, intraocular pressure increase may occur. The most frequent signs and symptoms were slurred speech, persistent mydriasis, unconsciousness/ unresponsiveness, hallucinations, kidney pain, dysphoria, "open eye dreams," hyperthermia, tremors, convulsions, psychomotor agitation, tachycardia and headache.[9] Also, it may produce psychiatric effects including suicidal ideas such as heroine.[10]

Based on the literature survey, there is only one case report related to use of TPC in Rome, Italy.[3] The only eye drops addiction in Turkey with a physiological dependence on cyclopentolate hydrochloride is reported according to self-declaration.[11] In another case study carried out by Agin et al. related with muscarinic effects and its toxic doses especially on children, after instillation cyclopentalat accidentally in both eyes in a total of only 12 drops. With this report, the random use of eye drops emphasized once again the sign of the danger.[12]

However, there is no literature concerning the determination of TPC from biological samples, except human aqueous humor determined in 2000 by Galmier MJ et al.[13] On the other hand only a few literatures were focused on the determination of this compound from drug formulations and as well as its major impurity detection method which is suitable for use in the quality control of TPC during its production.[14-16]

In this study, we present a validated method for TPC determination and quantitation by GC-MS in hair. This is the first encountered TPC abuse case in Turkey, which has determined by GC-MS method and the world's first report related the determination and quantitation of this compound from hair sample so far as is known .

MATERIAL AND METHOD

This study was evaluated under the scope of Ethics Committee Decision 10231, made by the Cerrahpaşa Medical Faculty Head of Ethics Committee on April 15, 2008.

Index Case

A 46 years old male patient was admitted to infirmary of the prison with complaints of nausea, vomiting and burning eyes for several times. In the last visit, besides these complaints, mydriasis, tachycardia, headache, hyperthermia, tremors were observed. Also the fundus observation could not be carried out detailed in the first intervention due to nistagmus and agitation. The biological samples of the patient have been transferred to our Forensic Toxicology Laboratory with any suspected drug or alcohol abuse by clinician. Toxicological screening results was negative both blood and urine for abused ordinary drugs or alcohol. Only TPC peak was obtained from screening test by GC-MS in hair.

Whole blood was analyzed using HS-GC-MS for the detection of ethanol and other volatile compounds. Moreover the blood and urine samples underwent our toxicological screening procedure using by GC-MS. From these analyses, the results for ethanol, other volatile compounds, pharmaceutical and psychotropic compounds were all negative.

Chemical and reagents

Tropicamide as European Pharmacopoeia (EP) Reference Standard was supplied from Sigma Aldrich. Nor-ketamine as internal standard (IS) was purchased from Cerilliant. Methanol, chloroform, n-propanol were purchased from Sigma Aldrich. N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), Sodium hydroxide (97%, pellets) and Hydrochloric acid (37%) were purchased from Sigma Aldrich. All the solvents used were of HPLC grade.

Extraction

Since hair sample of the patient was transferred to our laboratory unlabeled and blended, the segment analysis could not be conducted. Analysis was

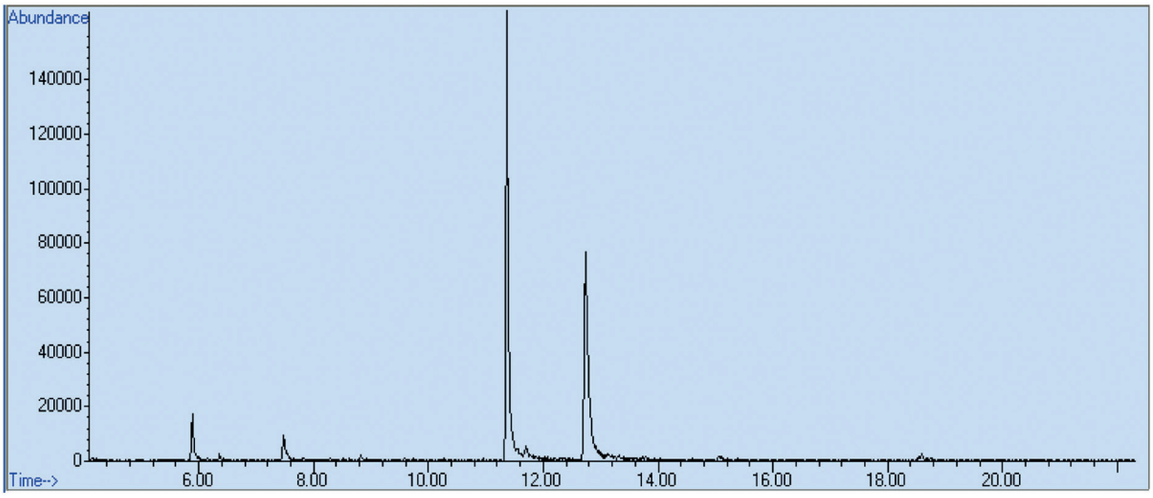

Figure 1. Chromatogram of the derivatized TPC standard (12.79+0.05 min) with nor-ketamine as IS (11.41+0.03 min).

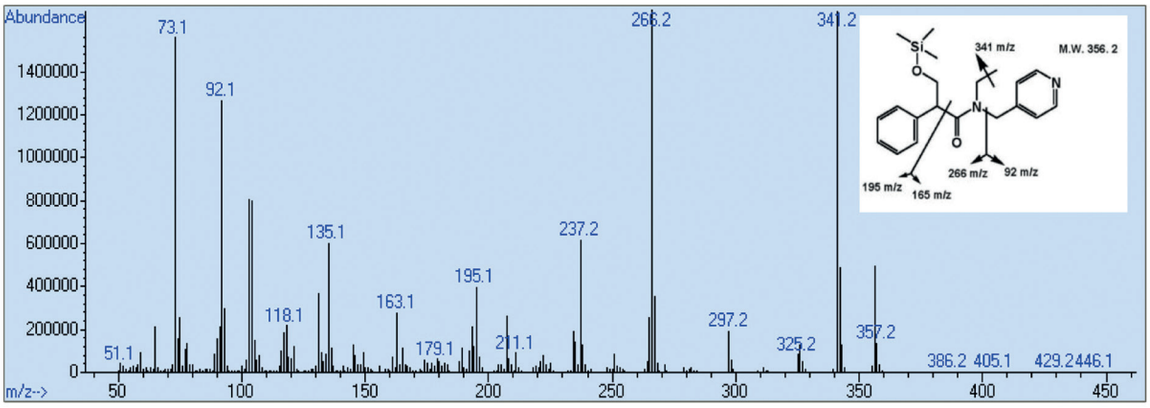

Figure 2. Full-scan EI mass spectra of the bis(trimethylsilyl)ether derivative of TPC and its fragmentation patterns.

performed on 150 mg hair weighed accurately. Hair was shampooed and rinsed with water, acetone and methanol, respectively. Sample was placed into a tube and 1 mL sodium hydroxide (0.001 M - pH 11.0) was added. After overnight incubation at 45 °C, the solution was neutralized with acidic solution (0.001 M hydrochloric acid - pH 3.0). After digestion, the liquid-liquid extraction process was performed triplicate by chloroform: 1-propanol (1:1, v/v) solvent mixture on hair sample at pH 9.6 by borate buffer. All pooled organic phases were evaporated to dryness under nitrogen flow at 45°C.

After evaporation, silylating agent (BSTFA containing 1% TMCS; 100 µL) was added to the residue and the vial was mixed, heated at 80°C for 30 min. After cooling, the derived solution was evaporated to dryness; the residue was re-dissolved in the presence of nor-ketamine (150 ng/mg) within 150 µL methanol.

GC-MS procedure

Method validation in hair was performed using the following GC-MS device and settings: HP 6890 (Agilent) and MS-Detector 5975B MSD (Agilent), equipped with a 30 m × 0.25 mm (i.d.) fused silica capillary column (HP-5MS 5% Phenyl Methyl Siloxane, film thickness 0.25 µm - Agilent Part No. 19091S-433). The oven temperature was programmed from 110° (1-min hold) to 250 °C at 15 °C/min (12-min hold). Total run time was 22.33 min. The temperatures of the injection port and interface were set at 250 °C and 230 °C, respectively with 1 µl injection volume. Split injection mode was used as 5:1 to minimize the matrix effect. Helium was used as a carrier gas at a final flow rate of 0.9 mL/min. The GC-MS method of this study was conducted in selected ion monitoring (SIM) modes with derivatization process. Ions used for identification were m/z 92, 266, 341, 357 (M^+). Quantitation was done only after derivatization with ion 92 for TPC. Ions used for IS were m/z 195, 166 and 131; quantitation ion for IS was m/z 166.

Method validation

Stock solution containing 2000 µg/mL TPC reference standard was prepared and stored at -20°C. Standard calibration curve was obtained using 15, 30, 75, 225,

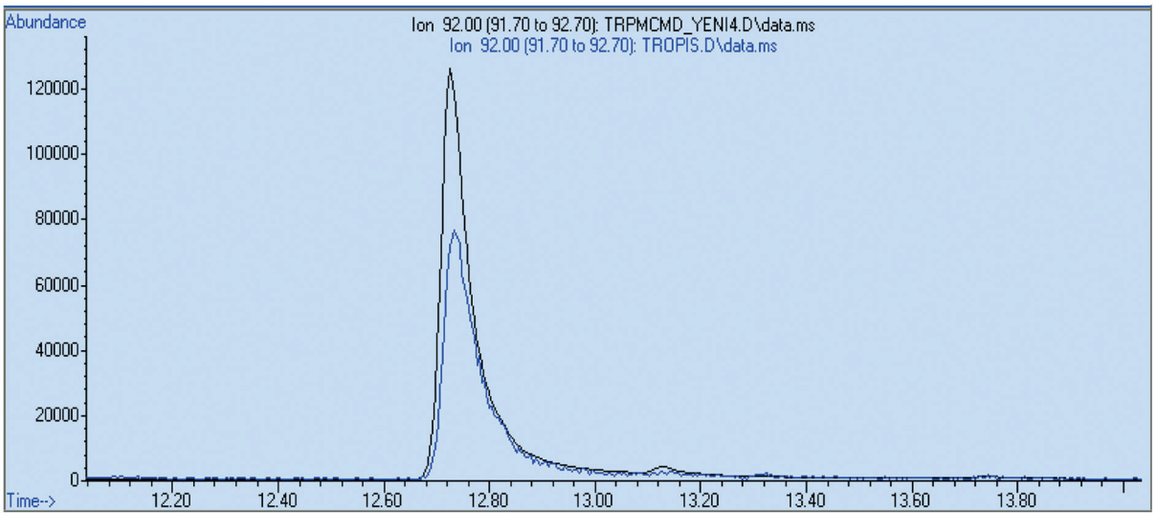

Figure 3. Overlaid chromatograms of the hair sample of the patient and spiked hair with TPC in SIM mode.

300, 600, 1200 ng per mg of the stock solution into 150 mg of drug free hair followed by derivatization after double extraction. Drug free hair sample was obtained from co-workers in our laboratory. TPC spiked hair samples with 225 and 300 ng/mg concentrations were also replicated 3 times to evaluate precision, accuracy and recovery. Limit of Detection (LOD) and Limit of Quantitation (LOQ) calculated according to the 135th Guideline of the US Food and Drug Administration (FDA).[17]

RESULTS

In the first step, TPC reference standard was derived with BSTFA containing 1% TMCS. The GC-MS chromatogram of the derivatized TPC standard and nor-ketamin (IS) was presented at retention time 12,79+0.05 min and 11.41+0.03 min, respectively (Figure 1).

The electron ionization (EI) mass spectrum of derived TPC was characterized by a molecular ion at m/z 357 (M^+). The most abundant fragment ion 266 m/z, corresponds to loss of CH_2-Pyrdine from the molecular ion. The second abundant fragment ion, 341 m/z, corresponds to loss of CH_3 from the molecular ion. The fragmentation pattern of the derived TPC was also shown in detail (Figure 2).

By adding increasing amounts of the reference standard to drug free hair, regression analysis of the calibration curve was obtained with 15-1200 ng/mg linear range. Linearity of the method was found R^2=0.998. Precision value was calculated by 3 replicates of 225 ng/mg hair sample and RSD% was found as 3.14. Recovery of the extraction method at 225 and 300 ng/mg were acceptable with 92.69% and 109.1%, respectively. Bias% was not more than 5.2 for accuracy. LOD and LOQ values were 2.88 and 8.73 ng/mg respectively.

Based on all these comparable data, quantitated TPC was determined 63.61 ng/mg in the prisoner hair according to calibration curve which acquired by adding incremental amounts of TPC standard in drug free hair (Figure 3). In this case, TPC was the unique compound determined based on the hair analysis by GC-MS, although antimuscarinic drugs are often abused in combination with other compounds such as alcohol, marijuana and opiates.[1] In addition to this, the symptoms of patient transferred to the clinic from the prison for many time, were compatible with other reported ones.[3]

Also, blood and urine are the most encountered specimens to report drug exposure. However, in some case related on drugs with short acting effect, hair samples are valuable to complement these analyses as hair can accumulate drug itself by roots. In the presented case, due to a frequent long delay between the prison to hospital, hair can be the only solution for a lack of confirmative evidence of abuse or any kinds of crime.

It is known that, there are considerable numbers of study conducted by hair analysis related with chronic drug poisoning and/or abuse except TPC. It has been used as a guide in some cases of forensic toxicology such as traffic accident, chronic legal or illicit drugs abuse, date rape, child abuse.[18-22]

Since analysis of polar compounds by GC-MS results in poor sensitivity and in peak tailing, derivatization methods have been extensively used to improve accuracy, reproducibility, and sensitivity. These methods by methylation, acetylation and silylation have frequently been used for identification and quantification of the drug accurately.[23] Human hair has advantages over other biological samples because

of its stability between sampling and analysis, easier collection and transportation. Hair is not evaluated as an alternative sample to blood or urine in drug abuse cases; it is only used as a complementary biological matrix.[24]

CONCLUSION

In Turkey, this drug was banned as of November 2012 and listed on controlled drug-list. It was concluded that it is necessary to develop new extraction methods for the determination of prescribed drugs as TPC from the biological materials since abuse of such substances are gradually increasing.

As a result, it is suggested that more studies need to be carried out with the cooperation of emergency clinician to confirm and better describe the new drug misuse. On the other hand, all medical experts should be rapidly informed about the alerting trend of misuse.

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* The authors declare that there are no conflicts of interest.

CORRESPONDING AUTHOR: Zeynep Türkmen İstanbul Üniversitesi Cerrahpaşa Tıp Fak. Adli Tıp Enstitüsü. Cerrahpasa, İstanbul, Türkiye zturkmen@istanbul.edu.tr

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