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Determination of tramadol in hair using solid phase extraction and GC–MS

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Abstract

Tramadol is a centrally acting synthetic analgesic with μ-opioid receptor agonist activity, it is a widely prescribed analgesic used in the treatment of moderate to severe pain and as an alternative to opiates. Tramadol causes less respiratory depression than morphine at recommended doses. Its efficacy and low incidence of side effects lead to its unnecessary prescribing in patients with mild pain. Tramadol was classified as a ''controlled drug'' long after its approval for use in Jordan. Analysis of drugs of abuse in hair has been used in routine forensic toxicology as an alternative to blood in studying addiction history of drug abusers. A method for the determination of tramadol in hair using solid phase extraction and gas chromatography–mass spectrometry (GC–MS) is presented, the method offers excellent precision (3.5–9.8%, (M) = 6.77%), accuracy (6.9–12%, M = 9.4%) and limit of detection 0.5 ng/mg. The recovery was in the range of 87–94.3% with an average of 90.75%. The calibration curve was linear over the concentration range 0.5–5.0 ng/mg hair with correlation coefficient of 0.998. The developed method was tested on 11 hair samples taken from patients using tramadol as prescribed by their physician along with other different drugs in treating chronic illnesses. Tramadol was detected in all hair samples at a concentration of 0.176–16.3 ng/mg with mean concentration of 4.41 ng/mg. The developed method has the potential of being applied in forensic drug hair testing. In Jordan, hair drug testing started to draw the attention of legal authorities which stimulated forensic toxicologists in recent years to develop methods of analysis of drugs known or have the potential to be abused.

Keywords: Forensic toxicology; Hair; Tramadol analysis; Drug of abuse; Jordan

1. Introduction

Detection of heavy metals in hair was first reported in 1857 [1]. In 1979, a new era in forensic toxicology started when Baumgartner et al. [2] reported the detection of opiates in hair, this was followed by several reports on the detection of other drugs in hair, e.g. cocaine [3,4], cannabis [5], selegiline [6], clonazepam [7] and phenobarbital [8].

Hair analysis is considered now a reliable qualitative technique for detecting exposure to the so called ''drugs of abuse'' even at low levels or after a long period of time, regardless of the pharmaceutical form of the drug and method of administration. The incorporation of the drug in hair is affected by many factors; the melanin content of the hair, the lipid solubility of the drug, its molecular size and shape, and the degree of ionization in the blood. Most drugs can be detected in hair long after their disappearance from body fluids, which makes hair analysis superior to other biological samples, e.g. urine and blood analyses [9]. The list of drugs that can be tested by this technique is rapidly expanding, in an effort to reduce illicit drug intake which is considered by many societies an antisocial or even criminal behavior.

The use of analgesics is not without risk of abuse, dependence, or addiction. The efficacy of tramadol and its low incidence of side effects increase these risks. In recent years, tramadol has become a widely prescribed

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analgesic. It is a centrally acting synthetic analgesic with μ-opioid receptor agonist activity [10]. It is an effective analgesic that is used in the treatment of moderate-to-severe pain [11] and as an alternative to codeine with less side effects. Tramadol hydrochloride is (±) *cis*-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol–HCl with a molecular weight of 299.9. The racemic tramadol is rapidly absorbed after oral administration and approximately 30% of the dose is excreted in urine as unchanged drug, whereas 60% of the dose is excreted as metabolites. The recommended daily dose of tramadol is between 50 and 100 mg every 4–6 h, with a maximum dose of 400 mg per day; the duration of the analgesic effect after a single oral dose of tramadol 100 mg is about 6 h with observed plasma half live of 6.3 h and a volume of distribution of 2.7 l/kg [12]. It is readily soluble in water and ethanol, with a pK_a 9.41 and log P octanol of 1.35 at pH 7.0.

Two factors are attributed to the misuse of tramadol locally. Drugs are usually dispensed without prescription as a general policy in Jordan, except those that are classified as ''dangerous or controlled drugs''. Tramaddol was registered in Jordan in 1995 as over the counter (OTC) drug. Such status prompted the competition between local drug manufacturers to produce it in large scale, followed by a post-marketing campaign in attempt to acquire a big market share of analgesic drugs. The second reason for over dispensing of tramadol is the substantially high bonus given to pharmacies and drug stores in Jordan to promote the selling of the more potent tramadol as alternative to the conventional and safer analgesics. As a result of some reports regarding its abuse, tramadol was classified as ''controlled drug'' in January 2001 after remaining outside the list of dangerous drugs for 6 years. That period of time was enough for many people to recognize its potent action, and this created a group of dependent users who will look for it at any cost.

Few reports in the literature discussed the issue of tramadol from the medicolegal point of view. Deaths involving tramadol overdose and death of impaired drivers have been published in literature [13–17]. On the other hand, there are several published reports on the use of solid phase extraction of tramadol from plasma, urine and blood [18–24] as a method of sample preparation. Our search for the analysis of tramadol in hair revealed the presence of two reports; one based on the use of liquid–liquid extraction [25] and the other on the use of a new and different type of solid phase extraction technology called headspace solid phase microextraction (HS-SPME) in hair sample preparation [26].

The aim of the study was to develop and validate a method for the analysis of tramadol in human hair with potential application in forensic toxicology cases using solid phase extraction and mass sepctrometery. The developed method was tested on hair samples taken from patients receiving tramadol at Jordan University Hospital (JUH); the analysis was carried out at the JUH toxicology laboratory and the Forensic Science Labaoratory.

2. Materials and methods

2.1. Standards and reagents

All organic solvents were high performance liquid chromatography (HPLC) grade obtained from LAB-SCAN, and all chemicals were reagent grade purchased from Merck (Pool, UK). Tramadol hydrochloride and papaverine hydrochloride are available at our toxicology laboratory. Buffers were prepared from HPLC grade reagents in deionized water. The solid phase extraction columns Isolute® HCX 200 mg, 10 ml capacity were purchased from (International Sorbent Technology, UK) and the solid phase extraction workstation was supplied by (Supelco, USA).

2.2. Hair samples

Eleven patients (five females and six males, aged 28–70-years old, the mean age was 45.8 years) were randomly selected from patients who were already being treated with tramadol for various medical illnesses and followed up in the surgical out-patient clinics at Jordan University Hospital. Information regarding the patients; age, sex, hair color, medical diagnosis and the drugs prescribed to the them during the course of their treatment are presented in Table 1. Blank hair was collected from two healthy volunteers known to be taking no medication.

Table 2 shows the dose and duration of tramadol administration. Hair samples were collected, within 1 month from stopping tramadol administration during their regular visit to the surgical out-patient clinic between January and May 2002. About (50–100 mg) of hair was collected close to scalp from the back of each patient's head. The collected hair sample was placed in a small plastic bag and a specific number was given for each sample, all were stored at ambient temperature (15–30 °C) until analysis. The consent of the patients was taken before sampling and their identity was kept anonymous. The sample collection was approved by the Institutional Review Board (IRB) of the Faculty of Medicine at Jordan University.

2.3. Hair decontamination

Hair samples were sonicated in ultrasonic bath once with 7.0 ml of 0.5 M sodium dodecylsulfate in de-ionized water for 10 min, the resulting wash was discarded. The next two sonications with 5 ml methanol each time were collected and evaporated to dryness at 40 °C under a stream of nitrogen, reconstituted with 500 μl methanol then added to the treated hair. The hair was allowed to dry overnight.

2.4. Solid phase extraction of hair

The dried hair was cut before being ground to fine powder using mortar and pestle. The powdered hair was placed in a screw capped glass vial. Twenty micrograms of the

Table 1
Age, sex, hair color, medical diagnosis and the drugs prescribed to the patients during the course of their treatment[a]

No.	Age	Sex	Color	Diagnosis	Other drugs given during the course of treatment
1	38	M	Black	Low back pain due to spine fracture	Pethidine, propoxyphene, codeine, paracetamol
2	37	F	Black	Behcet disease	Warfarin, steroid colchicine
3	43	F	Brown	Advanced Ca colon	Famotidine, heparin, Pethidine
4	61	M	Black	Advanced Ca colon	Pethidine, amikacine
5	47	M	Black	Multiple myeloma	Ranitidine, Bufferin®, amlodipine
6	47	M	Black	Multiple myeloma	Morphine, Aredia®, Diclofenac, Normacol®, amitriptyline
7	60	M	Black	Non-specific abdominal pain	Bufferin(r), nifidipine, insulin
8	30	F	Black	Ca colon	Warfarin, steroids, chlorpheniramine, digoxin, ranitidine
9	43	F	Black	Ca breast	Ranitidine, megastrol, metoclopramide, miconazol
10	28	M	Black	Ca colon	Cefuroxime, heparin
11	70	F	Black	Low back pain	Furosemide, atenolol, spironolactone, indomethacin, omeprazole

[a] All patients received tramadol.

powdered hair was weighed in a clean glass tube then 2 ml of 3 M HCL was added followed by 100 μl of the internal standard papaverine before overnight incubation at 60 °C. The sample was allowed to cool to room temperature, and the supernatant was removed following centrifugation at (3000 rpm for 10 min). The sample solution was adjusted to pH 9 using potassium borate buffer before extraction. HCX column was pretreated with 2.0 ml of methanol, 2.0 ml deionized water and 2.0 ml of 0.1 M potassium borate buffer pH 9. Then the sample was applied to the column. After washing the column with 2.0 ml of 1.0% acetic acid followed with 2.0 ml acetonitrile, tramadol was eluted with 2 ml of *n*-butyl chloride. The elution extract was evaporated to dryness at 40 °C under a gentle stream of nitrogen; the residue was reconstituted with 200 μl of methanol. One microliter was injected into the gas chromatography–mass spectrometry (GC–MS) system.

Table 2
Concentration of tramadol in hair samples collected within 1 month from stopping tramadol administration

No.	Dose	Duration	Concentration (ng/mg) in hair
1	100 mg × 2–3 M	7 months	5.06
2	100 mg × 1 M	2 months	3.94
3	100 mg × 2 M	12 days	0.21
4	100 mg × 2 M	10 days	0.176
5	100 mg × 2–3 T	3 months	1.26
6	100 mg × 1 M Or 1 T	2 months	5.95
7	100 mg × 2 M	6 weeks	0.83
8	50 mg × 2 T	1 month	1.1
9	50 mg × 2 T	1 week	8.65
10	50 mg × (2 × 3) T and 75 mg × 1 M Q 6 h	10 days 3 days	16.3
11	50 mg × 3 T	16 days	3.5

M: intramuscular injection; T: tablets.

2.5. Recovery, precision, accuracy, stability and limit of detection

To validate our method, tramadol was added to the solution obtained after the decontamination step of blank hair samples. The hair samples were obtained from persons who did not take tramadol or any other drug. The recovery of tramadol using the developed method was tested at four concentration levels of 0.5, 2.0, 8.0 and 20 ng/mg hair. Six samples at each concentration level were used to enable us to calculate precision and accuracy. The stability at 5 and 25 °C was tested on the extract of authentic hair samples. Furthermore, four blank hair samples spiked with decreasing amounts of tramadol were used to determine the limit of detection of the method.

2.6. Instrumentation

The determination of tramadol in hair was performed on GC–MS system consisting of a Varian star mode GC 3600 CX and a Varian SATURN 2000 mass spectrometer with electron-ionization mode at 70 eV. An HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm, cross linked 5% phenyl methyl siloxane) was used for separation (Hewlett Packard, Palo alto, CA, USA). Helium was used as a carrier gas at a constant flow of 1.2 ml/min. Injection was made in the splitless mode with splitless time of 60 s. The injector and interface temperature were 230 and 270 °C, respectively. The oven initial column temperature was held for 5 min at 60 °C then increased to 280 °C at a rate of 15 °C/min and held for 5 min at 280 °C. The total run of one injection was 25 min. The mass detector was operated at selective ion storage (SIS), to monitor the *m*/*z* of 262 and 58 for tramadol at 14–16 min and *m*/*z* 339 and 324 for the internal standard papaverine at 20–23 min. The retention times of tramadol and papaverine were (15.0 and 22.25 min), respectively. The response obtained by GC–MS on total ion current (TIC) was recorded.

3. Results and discussion

3.1. Validation of the developed method

To obtain a valid quantitative determination of tramadol the following analytical parameters: recovery, sensitivity, precision, accuracy and stability were determined as recommended by Causon [27]. The method offered excellent accuracy; precision expressed as CV% (3.5–9.8, M = 6.77) accuracy (6.9–12, M = 9.4) were obtained over the concentration range of 0.5–20 ng/mg hair. The results of precision and accuracy are summarized in Table 3. The ratio of peak area response of tramadol to internal standard for extracted and un-extracted was used in calculating recovery. Before GC–MS analysis, all samples (extracted and un-extracted) were evaporated to dryness, then reconstituted with 200 μl methanol. The recovery of tramadol from spiked hair was in the range of 87–94.3% with an average of 90.75% (Table 4).

The calibration curve for tramadol was obtained from the average of the two runs at each concentration, then the calibration curve was constructed by plotting drug concentration versus the peak area ratio of the drug/internal standard. The method was linear in the concentration range 0.5–8.0 ng/mg hair with correlation coffecient of 0.9981, $y = 0.0246x - 0.0041$, $r^2 = 0.9981$. A detection limit of 0.2 ng/mg hair was achieved when a decreasing concentration of tramadol spiked in blank hair reached a signal to back ground noise ratio of six. Tramadol was found to be stable in the extract of authentic samples with no significant loss = 4.0 and 9.5% for samples stored at 5 and 25 °C for 2 and 4 weeks, respectively.

Table 3
Precision and accuracy of the developed method after the vitro addition of tramadol standard to hair samples ($n = 6$)

Added amount	Mean concetration found (ng/mg)	CV (%)	Accuracy (%)
0.5	0.45	8.1	12.0
2.0	2.20	9.8	10.3
6.0	5.85	5.7	8.40
8.0	7.87	3.5	6.90
	Mean	6.77	9.4

Table 4
Recovery of tramadol from blank hair ($n = 6$)

Concentration (ng/mg)	Average ($n = 4$) recovery (%)
0.5	87.5
2.0	89.2
8.0	92.0
20	94.3
Mean recovery	90.75

The gas chromatography conditions used in separating tramadol and the internal standard offered a reasonable analysis time of less than 25 min for each run. The retention times of tramadol and internal standard were 15.0 and 22.25 min, respectively. The mass detector was operated to record TIC over the time range of 4.0–25 min. Fig. 1 shows the SIS for tramadol spiked in hair (a) and blank hair sample (b). The parent ions of tramadol were; m/z 264 and daughter ion m/z 58 at 15.0 min, and the internal standard ions; m/z 339 and 324 at 22.25 min.

Table 1 shows that 28 different drugs for different chronic medical illnesses had been given to the patients during the course of their treatment, beside the chemotherapy agents and analgesics which were usually prescribed for a relatively short period of time. The chromatograms obtained from patients hair samples showed no interferences at the retention times of tramadol or the internal standard (papaverine). The selective ion chromatogram of hair sample taken from patient no. 1 is presented in Fig. 1(c). This patient received tramadol (100–300 mg IM over 7 months) and other different analgesics like; Pethidine (meperidine), Dolostop® (paracetamol and propoxyphene) and Revacode® (codeine and paracetamol) during the course of treatment for low back pain due to fractured spine. The concentration of tramadol in his hair was 5.06 ng/mg.

In the early stage of our work on tramadol extraction, a solid phase extraction method described by Hadidi et al. [28] was applied to extract tramadol. The recovery was less than 40%. When the buffering solution was modified by using borate buffer, the recovery was significantly increased to approximately 65%. A recovery of above 90% was achieved when *n*-butyl chloride was used as elution solvent.

3.2. Tramadol concentration in hair

It is generally known that the concentration of drugs in blood and/or urine usually falls to levels that are difficult to detect after hours or days depending on the drug being used, even with the use of very sensitive detection methods. Our method was capable of detecting tramadol at a low concentration of 0.176 ng/mg to a high concentration of 16.3 ng/mg hair in authentic hair samples. The concentrations detected in all patients samples were within the range of the calibration curve.

It is known that migration of drugs to hair depends on their lipophilicity, the gradient concentration and the pH gradient on both sides. Since hair is an acidic medium, it was suggested that the pH gradient between blood (7.4) and hair (3.67) is of paramount value in the flux of acidic or basic drugs [29]. The presence of reasonable concentrations of tramadol in most of the cases is supported by the fact that hair incorporate basic drugs more than acidic ones (pK_a of tramadol is 9.41), our findings are in agreement with Eysseric et al. [30] who reported a tramadol concentration in the range of 2.8–97.93 ng/mg hair in fatal and non fatal cases.

Another contributing factor explaining the good incorporation of tramadol in hair is melanin content. It is known that black hair usually incoprorates basic drugs more efficiently because it contains higher melanin levels than white hair. In a controlled study, the incorporation of selegiline and its basic drugs metabolites (amphetamine and methamphetamine) showed an exponential relationship between basic drugs concentration and the melanin content in hair [6]. In our study, results of hair analysis showed a lack of a linear relationship between the dose of tramadol and its

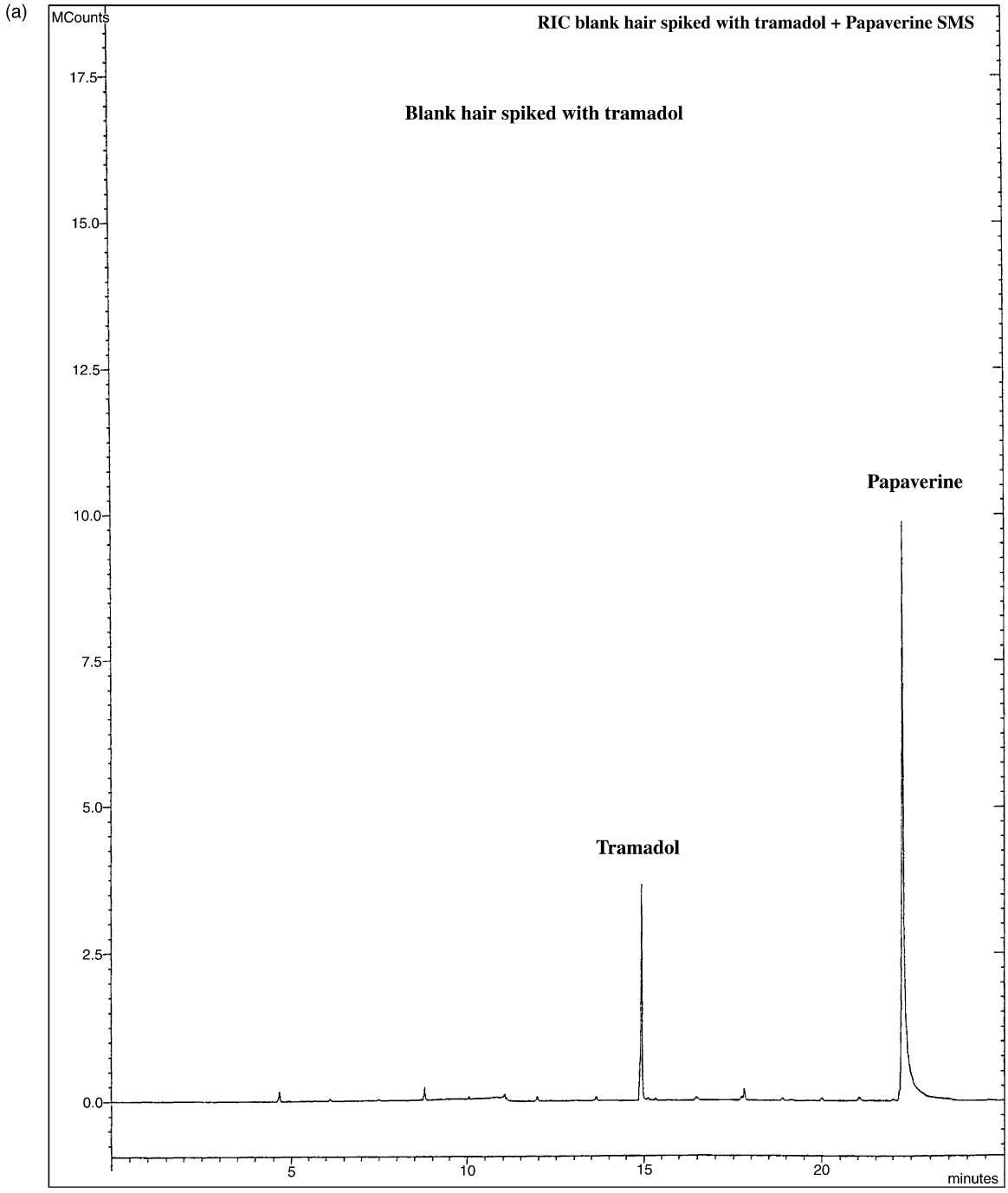

Fig. 1. The GC–MS/SIS chromatograms of: (a) blank hair spiked with tramadol hair; (b) blank hair and (c) hair sample taken from case no. 1.

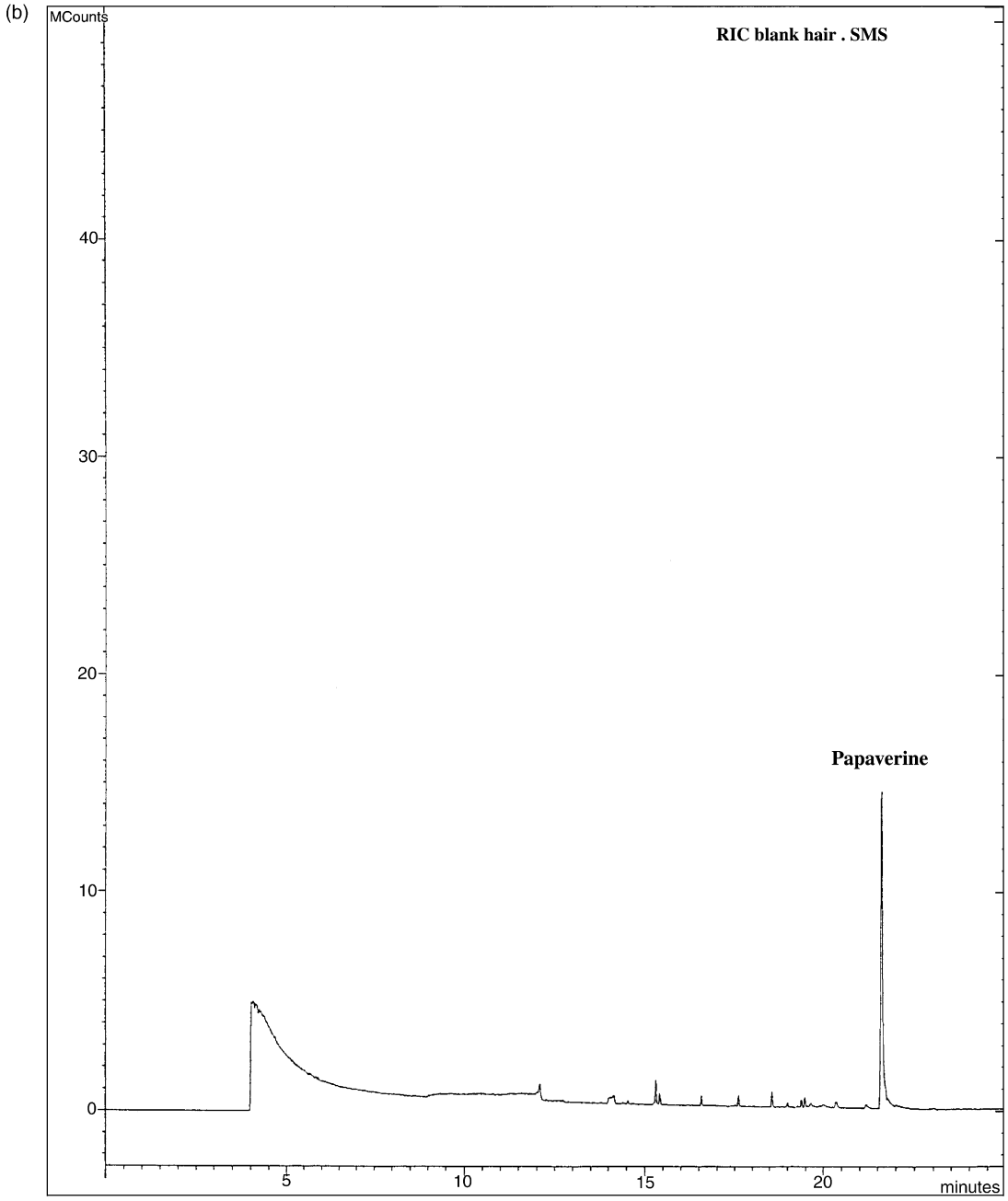

Fig. 1. (*Continued*)

concentration in hair for patients receiving almost the same dose for the same duration. Such finding is not surprising due to the fact that drug concentration in hair is affected by many known factors such as; the stage of hair growth and local blood supply, and unknown variables as; individual metabolic variation and the type and frequency of hair treatment. In our study population, another unknown variable should be considered and addressed and that is the effect of other drugs taken simultaneously with tramadol and the administration of tramadol before or after chemotherapy to some patients. A controlled study on the effect of chemotherapy agents and other drugs used in the treatment of chronic medical diseases on the incorporation of drugs in hair and the correlation between blood and hair tramadol concentration will be performed.

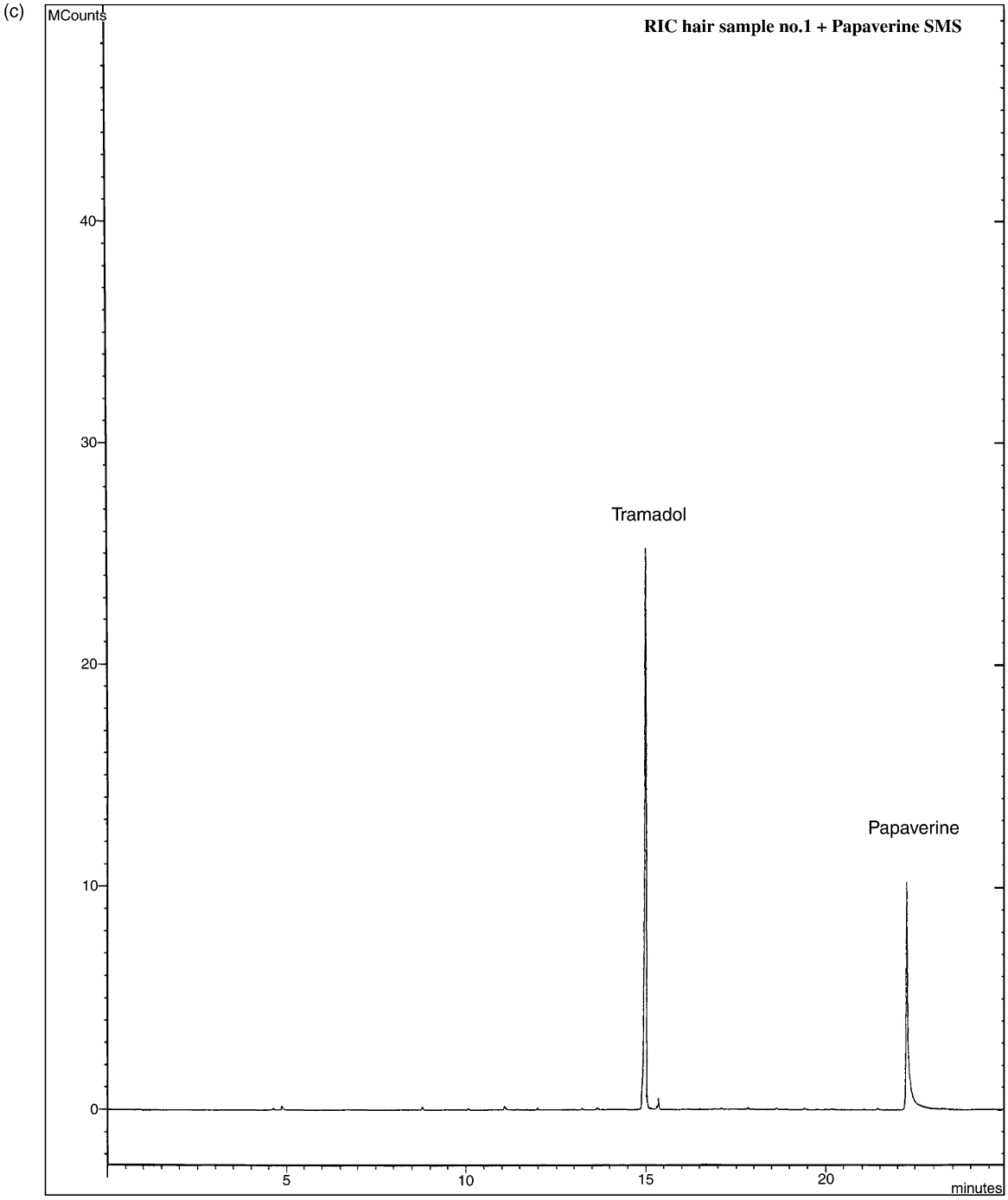

Fig. 1. (*Continued*).

4. Conclusion

We conclude that the developed method which was based on the use of solid phase extraction and gas chromatography–mass spectrometry, has excellent sensitivity and specificity in detecting tramadol even when multi-drugs have been prescribed to patients to treat different chronic medical conditions. Regardless of the known or unknown factors affecting the incorporation of drugs in hair, tramadol was still detectable in all patients hair samples. This method supports the role of forensic toxicologists in the diagnosis of tramadol use or misuse and the follow-up of individuals even

during their medical treatment. The alarming sign of increasing abuse of tramadol in Jordan should encourage law enforcement to accept the valuable results obtained from hair analysis in confirming tramadol misuse.

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