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ORIGINAL ARTICLE

Rapid analysis of urinary opiates using fast gas chromatography–mass spectrometry and hydrogen as a carrier gas

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Abstract A sensitive and specific fast gas chromatography–mass spectrometry (FGC–MS) analytical method using hydrogen as a carrier gas is developed for the rapid simultaneous determination of morphine, codeine, hydrocodone and hydromorphone in human urine. Urine samples were spiked with deuterated internal standards, morphine-d3, codeine-d3, hydrocodone-d3 and hydromorphone-d3, subjected to acid hydrolysis, treated with hydroxylamine to convert the keto-opiates to oximes and then extracted using a positive pressure manifold and silica based solid phase extraction columns. The extracts were derivatized using BSTFA with 1% TMCS.

Gas chromatographic–mass spectrometric analysis was performed in electron ionization mode by selective ion monitoring, using hydrogen as a carrier gas, a short narrow bore GC capillary column, and fast temperature program, allowing for a rapid analytical cycle to maximize the instrument time for high throughput laboratories. While maintaining specificity for these drugs, concentrations in human urine ranging from 50 to 5,000 ng/mL can be measured with intraday and interday imprecision, expressed as variation coefficients, of less than 2.3% for all analytes within a run time of less than 3.5 minutes.

1. Introduction

Codeine and morphine are naturally occurring alkaloids derived from the seedpod of the opium poppy while hydrocodone and hydromorphone are semi-synthetic opiate derivatives and heroin is a diacetyl derivative of morphine. The opiates are classified as narcotic analgesics with codeine, hydrocodone and hydromorphone additionally having antitussive properties. Heroin, a Schedule I Controlled Substance in the United States, is generally administered by intravenous or subcutaneous injection, or less frequently by smoking or nasal insufflation. Morphine, codeine, hydrocodone and hydromorphone are Schedule II Controlled Substances. Morphine can be administered by intravenous, intramuscular, or oral routes, while codeine, hydrocodone and hydromorphone are usually administered orally. Pharmacologic effects of the opiates, in addition to analgesia,

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include euphoria, sedation, pupillary constriction, respiratory depression, and constipation at therapeutic dosages. Overdose of morphine/heroin can cause coma[1] and death by cardiopulmonary collapse[2] and an overdose of codeine can cause unconsciousness and convulsions; death may result from respiratory failure.[3] Toxic effects of hydrocodone and hydromorphone include stupor, muscle flaccidity, respiratory depression, hypotension, cold and clammy skin and coma.[4]

Morphine is rapidly absorbed in the body. Plasma peak levels following an oral dose occur after 15–60 min, and following injection can occur after 15 minutes.[5] Extensively metabolized by the liver, only 2–12% is excreted as unchanged drug, while 60–80% is excreted as morphine-3-glucuronide. The elimination half-life of morphine ranges from 1 hour to 8 hours. Heroin is rapidly metabolized (plasma half-life is approximately 3 minutes), first to monoacetylmorphine and further to morphine. Morphine is the primary metabolite excreted in urine after heroin abuse. Approximately 7% is excreted as unchanged morphine and 50–60% as glucuronides. Additionally, the specific heroin metabolite, 6-monoacetylmorphine (6-MAM) may be detected in urine 4 h–8 h after the ingestion of heroin. Codeine is also rapidly absorbed and metabolized following an oral dose, principally to codeine-6-glucuronide, with 10%–15% metabolized to morphine and norcodeine; 5%–17% of the codeine dose is excreted as a free drug.

Hydrocodone is more toxic than codeine and metabolized in the liver with most metabolites being pharmacologically active. About 26% of a single dose is eliminated in the 72 h urine as unchanged drug. Hydromorphone is metabolized in the body to hydromorphone-3-glucuronide and hydromorphol. About 6% of an average dose is excreted as free and 30% as conjugated hydromorphone in the 24 h urine. Opiates may be detected in the urine for 24 h–72 h following ingestion.

The opiates/opioids are encountered frequently in forensic toxicology as they are heavily prescribed and abused. The recent upsurge in pain clinics throughout the United States and the dispensing of large quantities of oxycodone and hydrocodone further highlights the forensic importance of this drug class.[6–8] According to the Centers for Disease Control and Prevention (CDC), 100 people in the United States die from drug overdoses every day, and death rates as a result of drug overdoses have more than tripled since 1990. The CDC also reports that nearly three out of four prescription drug overdoses are caused by opiates.

Drug testing for opiates under the Mandatory Guidelines for Federal Workplace Drug Testing Programs[9] in the United States currently requires immunoassay screening and confirmation by gas chromatography–mass spectrometry (GC–MS) or the recently approved technique of liquid chromatography–mass spectrometry (LC–MS) for morphine and codeine. Substance Abuse and Mental Health Services Advisory (SAMHSA) is proposing the addition of hydrocodone and hydromorphone to this mandatory testing program. The immunoassays available for opiate testing have variable cross-reactivity to codeine, morphine, and other opiates.[10] Detection and quantitation of keto-opiates like hydrocodone, hydromorphone, oxycodone, and oxymorphone are desirable (1) because of their potential interference with the measurement of codeine and morphine and (2) because of their increasing potential for abuse.

Recent advances in instrumentation like liquid chromatography with tandem mass spectrometry (LC–MS–MS) have demonstrated simultaneous detection of naturally occurring opiates and their synthetic derivatives such as hydrocodone and hydromorphone in various matrices,[11–14] but this instrumentation is more expensive than traditional electron impact gas chromatography (EI-GC)–MS systems and may be cost-prohibitive in many toxicology laboratories.

Several GC–MS methods have been developed for the analysis of codeine, morphine, and/or other opiates. The extraction, derivatization, and detection details of many of these methods have been published in review articles.[15,16] Stability and characteristics of various derivatives used for opiate anal-ysis[17,18] as well as the hydrolysis efficiency of acid and enzymes have also been studied.[19]

Problems encountered in GC–MS methods for simultaneous analysis of morphine and codeine include interference from keto-opiates like hydrocodone, hydromorphone, oxyco-done and oxymorphone in the analysis of codeine and morphine, incomplete derivatization, instability of derivatives, poor chromatography, inefficient hydrolysis, especially in case of enzyme hydrolysis, and extended run times. Techniques to improve separation of these opiates include pretreatment with borohydride,[20] sequential derivatization,[21] and multiple ramp temperatures.[22] Several methods have been reported that utilize dual derivatization with hydroxylamine to form oxime derivatives of the keto-opiates followed with BSTFA treat-ment.[23–26] Others have used methoxyamine and propionic anhydride with pyridine as a catalyst[27] for derivatization or a three step derivatization with methoxyamino/propionyl/ TMS groups.[28]

The method presented here is a modification of previously published methods that utilized hydroxylamine, and was developed to address the high throughput laboratory needs for faster turnaround times that the previously reported GC–MS methods did not address. The procedure includes acid hydrolysis of urine samples followed by reaction with hydroxylamine, extraction on solid-phase columns, and derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Codeine, morphine, hydrocodone, and hydromorphone are separated using a short, narrow bore capillary column, fast temperature programing and hydrogen as a carrier gas, within 3.5 min, and without cross-interference. Quantitation was performed with deuterated internal standards in selected ion monitoring (SIM) mode.

2. Materials and methods

2.1. Reagents and consumables

Certified drug-free urine was obtained from UTAK Laboratories (cat# 88121-CDF). BSTFA with 1% TMCS was purchased from Pierce Chemical Company (Rockford, IL). Hydroxylamine hydrochloride was obtained from Sigma Chemical Company (cat# H-9876). Sodium phosphate, mono-basic, monohydrate and sodium phosphate, dibasic, anhy-drous (cat# 3818–01 and 38828–01) were purchased from J.T. Baker (Phillipsburg, NJ). Hydrochloric acid, acetic acid, ammonium hydroxide, methanol, dichloromethane, and isopropyl alcohol were obtained from Spectrum Chemicals (Gardena, CA). All solvents were of HPLC grade or better and all chemicals were of ACS grade. Solid phase extraction columns (Clean Screen) were obtained from United Chemical Technologies, Bristol, PA. Gas chromatographic capillary column (CP-SIL 5CB, cat# CP7684) was obtained from Agilent, Inc. (Lake Forest, CA).

2.2. Calibrators and controls

The deuterated internal standards, codeine-D3, morphine-D3, hydrocodone-D3 and hydromorphone-D3 (1 mg/mL in methanol) as well as the unlabelled drugs (1 mg/mL in methanol) codeine, morphine, hydrocodone and hydromorphone were obtained from Cerilliant (Round Rock, TX). The mixed internal standard solution containing all the deuterated analogs was prepared in methanol at a concentration of 2000 ng/mL. Calibrators were prepared from a mixed working stock solution of codeine, morphine, hydrocodone and hydromorphone in drug-free urine at a concentration of 5000 ng/mL. Stock reference material (1 mg/mL in methanol) of each opiate was also obtained from an alternative manufacturer (Alltech, Deerfield, IL) for preparation of low and high positive controls, at concentrations of 250 and 2000 ng/mL, respectively, in drug-free urine. A separate carryover control was prepared at 20,000 ng/mL in drug-free urine. The solutions were stored at 20 °C when not in use. Drug-free negative, low and high positive controls and the carryover control were included in every batch for analysis.

2.3. Preparation of working reagents

6 N hydrochloric acid: 400 mL of de-ionized water was added to a 1 L volumetric flask and 500 mL of concentrated hydrochloric acid was slowly added to the flask. The contents were diluted to 1 L with de-ionized water.

7.4 N ammonium hydroxide: 200 mL of de-ionized water was added to a 500 mL volumetric flask and 250 mL of concentrated ammonium hydroxide was slowly added to the flask. The contents were diluted to 500 mL with de-ionized water.

0.1 M phosphate buffer: pH 6.0: 2.93 g of sodium acetate and 1.62 mL of glacial acetic acid were added to a 500 mL volumetric flask containing 400 mL of de-ionized water and then diluted to 500 mL with de-ionized water. The pH was adjusted to 4.5 ± 0.1.

0.1 M acetate buffer: pH 4.5: 12.14 g of monobasic sodium phosphate and 1.7 g of dibasic sodium phosphate were added to a 1 L volumetric flask and diluted to 1 L with de-ionized water. The salts were dissolved by shaking the flask.

10% hydroxylamine hydrochloride: 10 g of hydroxylamine hydrochloride was added to 100 mL volumetric flask and diluted to 100 mL with de-ionized water.

Elution solvent: Elution solvent was prepared by adding 2 mL of concentrated ammonium hydroxide to 20 mL of isopropyl alcohol, mixing and adding 80 mL of dichloromethane.

2.4. Sample preparation

Calibrators were prepared from the 5000 ng/mL mixed work-ing stock solution in 1 mL drug-free urine at concentrations of 100, 250, 500, 1000 and 5000 ng/mL for all analytes. 0.2 mL of internal standard (mixed deuterated opiates) solution was added to all tubes. The samples were subjected to acid hydrolysis before solid phase extraction to release the free drugs from their conjugates.

2.5. Hydrolysis method

1 mL of 6 N hydrochloric acid was added to 1 mL urine. The contents were mixed and the tubes were incubated at 120 °C for 30 min. The samples were allowed to cool to room temperature before extraction.

2.6. Conversion of keto-opiates to oximes

0.5 mL of 7.4 N ammonium hydroxide and 0.5 mL of 10% hydroxylamine solution were added to the specimens in order to convert the keto-opiates to their oximes prior to extraction and to eliminate interference with morphine and codeine. The specimens were vortexed and incubated at 70 °C for 15 min. Additional 0.5 mL aliquot of 7.4 N ammonium hydroxide was added after allowing the samples to cool to room temperature to bring the pH between 6 and 8.

2.7. Extraction procedure

The samples were extracted using solid phase extraction. 2 mL of 0.1 M phosphate buffer (pH 6.0) was added to all the specimens before extraction. The extraction columns were conditioned by sequentially passing through 2 mL of methanol, 2 mL of de-ionized water and 2 mL of 0.1 M phosphate buffer (pH 6.0). Samples were then loaded on to the extraction columns and washed sequentially with 3 mL of de-ionized water, 3 mL of acetate buffer (pH 4.5) and 3 mL of methanol. The columns were dried for 5 min under air pressure (25–30 psi) before eluting the drugs with 2 mL of freshly prepared elution solvent (methylene chloride: isopropanol: ammonium hydroxide, 80:20:2 v/v). The solvent was evaporated to dryness under a gentle stream of air at room temperature, followed by the addition of 50 μL of the derivatizing reagent (BSTFA with 1% TMCS) and 50 μL ethyl acetate. The tubes were briefly vortexed and incubated at 70 °C for 20 min. The samples were transferred to auto sampler vials after cooling to room temperature, and subsequently injected into the GC–MS system.

2.8. Gas chromatography–mass spectrometry

A Shimadzu QP 2010S gas chromatograph with a 220 V oven, capable of fast temperature programming, coupled to an inert mass selective detector, operating in electron impact mode, was used for analysis. Hydrogen generated by a Parker Balston hydrogen generator (Model# 920071) was used as a carrier gas at a constant flow of 1 mL per minute. The GC column dimensions were 10 m 0.15 mm, film thickness 0.12 μm, and the injector was operated in splitless mode at 250 °C. The oven temperature program was initiated at 150 °C (held for 0.5 min), and ramped to 300 °C at a rate of 40 °C/min. The selected ions monitored for the deuterated internal standard and all the analytes, along with their retention times, are indicated in Table 1.

2.9. Data analysis

Calibration was carried out using least squares linear regression analysis over a concentration range of 100–5000 ng/mL.

Shimadzu Solutions software automatically calculated the peak area ratios of target analytes and the internal standard for each calibrator concentration. The data was fit to a linear regression curve with equal weighting. Morphine, codeine, hydromorphone and hydrocodone were confirmed quantita-

tively with three ions monitored for each compound and resulting ion ratios were required to be within ± 20% of those of the 100 ng/mL calibration standard for acceptance. In addition, a retention time window of ± 2.5% of the mean calibrator retention time was required for acceptance of control and unknown peaks. Quantitation of positive controls was required to be within 20% of the established mean for each control for analytical run acceptance.

2.10. Specificity

Five drug-free urines were collected from laboratory volunteers. A 2 mL aliquot of each urine sample was extracted and analyzed as described, in order to detect any potential interference from endogenous compounds in the urine. Additionally, 2 mL of drug-free urine was spiked with 10,000 ng/mL of cathinone, diphenhydramine, doxylamine, desmethylsegiline, ephedrine, fenfluramine, methylphenidate, methylaminorex, mescaline, amphetamine, methamphetamine, MDMA, MDA, MDEA, MBDB, BDB, methoxymethamphetamine, methoxyamphetamine, pseudoephedrine, phentermine, phenmetrazine, propoxyphene phenylpropanolamine, nor-propoxyphene, dihydrocodeine, norcodeine, normorphine, cis-tramadol, methadone, N-desmethyl-cis-tramadol, methadone, dihydromorphine, oxymorphone, meperidine, normeperidine, buprenorphine, norbuprenorphine, dextro-methorphan, meprobamate, methylphenidate, fentanyl, nor fentanyl, nalorphine, naltrexone, naloxone, ketamine, nor ket-amine, nordiazepam, temazepam, 2-hydroxy-ethyl-flurazepam, 7-aminoflunitrazepam, oxazepam, alpha-hydroxy-alprazolam, lorazepam, 7-aminoclonazepam, alpha-hydroxy triazolam, PCP, butalbital, secobarbital, amobarbital, phenobarbital, pentobarbital, benzoylecgonine, cocaine, trazodone, hydrox-ybupropion, fluoxetine, norfluoxetine, pentazocine, dextro-phan, citalopram, sertraline, nor-sertraline, butorphanol, carbamazepine, nalbuphine and paroxetine. The spiked urine samples were extracted and analyzed as described to access potential interference in terms of co-elution and quantitative accuracy.

2.11. Linearity and sensitivity

Drug-free urine was spiked with morphine, codeine, hydromorphone and hydrocodone at 50, 100, 250, 500, 1000, 2500, 5000, 10,000, 20,000 and 50,000 ng/mL to determine the range of linearity. The limit of quantitation (LOQ) of the method was defined as the lowest concentration of analyte in the sample that could be quantitatively detected within ± 20% of the expected value, with a signal-to-noise ratio (peak height) in excess of 10, and be within retention time (±2.5% of the calibration standard) and ion ratio (±20% of the calibration standard) acceptance criteria. The limit of detection (LOD) was defined as the lowest concentration of an analyte that could be qualitatively detected and maintain retention time, ion ratio, and chromatography acceptance criteria. Five replicates of each concentration level were analyzed to determine the LOD and LOQ of the assay.

Table 1 Ions used for SIM analysis of opiates and the internal standards used.

Analyte	Ions		Retention time
	Target ion	Qualifier ions	
D3-codeine	374	346	2.73
Codeine	371	343, 234	2.76
D3-morphine	432	417	2.93
Morphine	429	414, 287	2.95
D3-Hydrocodone	300	389	3.06
Hydrocodone	297	386	3.07
D3-hydromorphone	358	447	3.12
Hydromorphone	355	444, 429	3.13

2.12. Imprecision

Imprecision was expressed as the percent relative standard deviation for a statistically significant number of samples. Imprecision, in terms of coefficient of variation, was determined at three levels for each analyte. The specimens, fortified with all opiates at concentrations of 100, 250 and 500 ng/mL were prepared. Five replicates of each concentration were analyzed according to the described procedure within a day (interday imprecision) and for five consecutive days (intraday imprecision).

2.13. Stability of opiates in urine as derivatized extracts

Stability of the derivatized extracts was investigated. Previously analyzed sample vials containing derivatized extracts were left on the instrument auto sampler exposed to light and room temperature for a period of two days and then re-analyzed. Any change in the concentration between the days was noted.

2.14. Application to authentic specimens

Specimens routinely received in the laboratory screening positive for opiates by enzyme immune assay were analyzed using the described protocol. A comparison was drawn between the quantitative results obtained from the conventional GC–MS method utilizing a 30 m capillary column and FGC–MS (Fast GC–MS) to determine the suitability of the new method.

3. Results and discussion

3.1. Method validation

The method for the detection of opiates was validated using drug-free urine fortified with various concentrations of the analytes as described. Authentic biological urines, with high drug concentrations, were diluted into the linear range of the assay.

3.2. Specificity

No endogenous interference was noted from biological drug-free urine extracts and no exogenous interference from the spiked drugs was noted in the assay.

3.3. Linearity and sensitivity

Linearity was observed from 100 to 5000 ng/mL for all opiates analyzed. The linear regression equations of calibration curves,

Table 2 Limits of detection, limits of quantitation and calibration curve equations for opiates in urine.

Analyte	LOD* (ng/mL)	LOQ* (ng/mL)	Linear equation** (y = response ratio)	Correlation, r^2
Codeine	50	100	$y = 1.160x - 0.0752$	0.999
Morphine	50	100	$y = 1.580x - 0.1650$	0.999
Hydrocodone	50	100	$y = 1.420x - 0.1650$	0.998
Hydromorphone	50	100	$y = 0.470x - 0.0458$	0.999

* Reported LOD/LOQ values are based on five determinations at each concentration.
** Linear equation based on six concentrations ranging from 100 to 5000 ng/mL.

Table 3 Inter-day ($n = 5$) and intra-day ($n = 5$) imprecision for the determination of opiates in urine.

Analyte	CV (%) at 100 ng/mL		CV (%) at 250 ng/mL		CV (%) at 500 ng/mL	
	Intra	Inter	Intra	Inter	Intra	Inter
Codeine	0.7	0.7	0.5	1.1	0.6	1.5
Morphine	1.9	2.3	0.7	1.7	0.9	1.2
Hydrocodone	1.5	1.3	1.0	0.8	0.5	1.1
Hydromorphone	1.4	2.3	1.5	2.0	1.3	1.4

Table 4 Stability of the derivatized opiate extracts after 24 h and 48 h.

Analyte	Conc. spiked	Conc. at 0 min	Conc. at 24 h	Conc. at 48 h	Mean	STD	CV%
Codeine	100	100	99.8	100.1	99.97	0.12	0.12
	200	200.6	199.7	200	200.10	0.37	0.19
	500	502.2	502.4	502.5	502.37	0.12	0.02
Morphine	100	100	99.9	99.8	99.90	0.08	0.08
	200	200.6	200.4	200.08	200.36	0.21	0.11
	500	502.7	502.4	501.7	502.27	0.42	0.08
Hydrocodone	100	100	100	99.6	99.87	0.19	0.19
	200	200.1	199.9	200.5	200.17	0.25	0.12
	500	504.4	501.9	502.3	502.87	1.10	0.22
Hydromorphone	100	99	100	99.8	99.60	0.43	0.43
	200	200.3	200.3	200.05	200.22	0.12	0.06
	500	502.9	502.5	502.2	502.53	0.29	0.06

LOD, LOQ, and correlation coefficient for all opiates analyzed are presented in Table 2.

3.4. Imprecision

Interday and intraday imprecision expressed as coefficient of variation (CV) for all analytes was less than 2.3%. Values at three different concentrations for all four analytes are presented in Table 3.

3.5. Stability of opiates in urine as derivatized extracts

Derivatized extracts were found to be stable at room temperature for up to 48 h. Mean recovery, standard deviation and CVs from the stability studies are presented in Table 4.

3.6. Application to authentic specimens

The newly developed procedure was applied to 76 clinical specimens previously analyzed by the laboratory using a more conventional method utilizing a 30 m GC column (DB-5 with 0.25 mm i.d.) and helium as the carrier gas. The results obtained with the two methods were compared and are shown in Table 5. It is evident from the table that the results obtained from the two methods are reproducible.

The sample analysis time with the conventional method utilizing a 30 m column and helium as the carrier gas was 10 min with the cycle time being 15 min whereas with the new method utilizing fast chromatography achieved by using a much shorter, narrow bore column and hydrogen as the carrier gas, the sample analysis time was reduced to 3.5 min/sample with the cycle time being 6 min. Sample throughput was more than doubled by utilizing this method thereby optimizing the instrument productivity. A typical total ion chromatogram (TIC) showing separation of all four opiates is presented in Figure 1

Opiate testing for morphine and codeine is performed routinely in forensic urine drug-testing laboratories in an effort to identify illicit opiate abusers. In addition to heroin, the 6-keto-opioids, including hydromorphone and hydrocodone, have high abuse liability and are self-administered by opiate abusers. This makes opiates one of the most frequently tested and detected substances in drug testing cases. Having a fast, high throughput method for quantitation in biological fluids is much needed for this high volume assay. The current

Table 5 Clinical sample results from conventional verses fast method.

Sample #	Conventional method				Fast method			
	Codeine	Morphine	HYC	HYM	Codeine	Morphine	HYC	HYM
1	10,080	1081			9879	958		
2	281	1867			258	1755		
3			1201				1194	
4		100	101	107		104	103	111
5			1688	165			1584	177
6			161.8	456			142	417
7			94	872			98	872
8		362				334		
9								
10		536				470		
11			1087	1546			979	1586
12			1893	1184			1783	1170
13			748	168			665	181
14			780	284			718	318
15			324.6				292	
16	215				202			
17	3831	508			3899	479		
18			2197	188			2084	221
19	10,030	1680			8979	1525		
20			115	272			121	240
21								
22			180	196			205	179
23			289	140			296	147
24			599	160			626	142
25								
26			95				110	
27		276	398	271		254	426	262
28				3546				3706
29			975	157			1010	133
30			306	202			321	194
31			5454	3099			5400	3163
32		560				548		
33		2357				2496		
34	2580	188			2897.6	164		
35			464	159			494	122
36		10,895		317		11,693		292
37		1278				1538.9		
38			313	138			329	151
39		673				764		
40		144				125		
41			1214	399			1301	417
42			467	108			477	98
43		251				225		
44	557				620			
45			885	179			818	158
46			55	106			69	92
47	2140	230			2419	206		
48								
49	161	287			162	327		
50	9346	1301			9762	1424		
51		946		73		1057		70
52								
53	1072	7552		147	1124	8419		136
54			1543	1266			1549	1394
55		4901	6577	1533		5538	6285	1569
56	137	3625			136	4107		
57	6112	1249			6407	1373		
58			271	332			270	313
59			1655	623			1674	644
60			7671	2552			8038	2693
61				1457				1207
62			234				230	
63			139				135	

Table 5 (*continued*)

Sample #	Conventional method				Fast method			
	Codeine	Morphine	HYC	HYM	Codeine	Morphine	HYC	HYM
64		4886		268		4388		247
65		10,074		1677		11,663		1637
66			4519	93			4104	88
67			253	196			248	180
68			625	584			616	567
69			264	335			250	309
70	2033	3259	241	413	2119	4174	219	422
71	201	605	289	514	200	689	279	510
72	12,542	9018		421	12,724	11,429		398
73				3883				3716
74			1647	567			1619	554
75			73	150			62.2	137
76			1501	735			1397	717

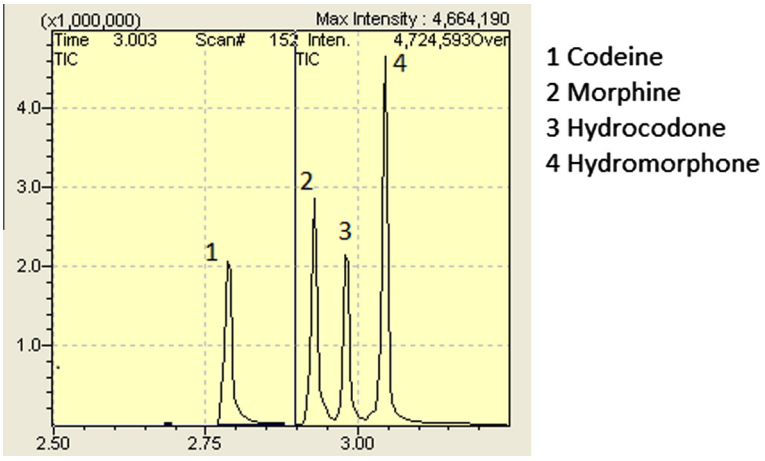

Figure 1 Total ion chromatogram.

method was developed for urine analysis for high volume laboratories but can easily be modified for other matrices.

4. Conclusions

Minor modifications in the previously published analytical method coupled with a very short, narrow bore GC capillary column and the use of hydrogen as the carrier gas led to extensive improvement in the instrument throughout without sacrificing sensitivity and specificity of analysis.

Funding

None.

Conflict of interest

No conflict of interest.

Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

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