GC-FID İLE EROİN, MORFİN, KODEİN VE 6-MONOASETİLMORFİN TAYİNİ VE METODUN VALİDASYONU

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Güvenilirlik ve itibar mahkemelerce de önemli bulunmaktadır. Bu sebeple adli laboratuarlarda kullanılan metotların validasyonu ve devamlı iyileştirmesi oldukça önem kazanmaktadır.

ÖZET

Amaç:

Bu çalışmamızda GC/FID cihazı ile uyuşturucu örneklerinde eroin (diasetilmorfin), morfin, kodein ve 6 asetilmorfin'in kantitatif tayini için kullanılan metodun validasyonu yapılmıştır

Yöntemler:

Çalışmamızda GC/FID Agilent 6890N cihazı ve Chemstation yazılımı, non polar yapıda %5 fenilmetilpolisiloksan analitik kolonu (DB-5: 10,0m x 100 µm x 0,17 µm) kullanıldı. Kullanılan standart madde ve solusyonlar ise Diklorometan-isopropilalcol (1: 1), Metanol, EroinHCl.H2O, KodeinHCl, MorfinHCl, 6-Monoasetilmorfin HCl, Parasetamol, kafein and n-dokosan referans ve internal standart olarak kullanılmıştır. Metodumuzda taşıyıcı gaz olarak Helyum kullanılmıştır.

Enjeksiyon sıcaklığı 280 C ve enjeksiyon hacmi 1 µl'dir. Tekrarlanabilirlik çalışması 4 operatör ile yeniden üretilebilirlik çalışması 3 operatör ile 29 gün için hesaplandı.

Bulgular:

Çalışmamızda eroin (0.01-2.00mg mL-1), kodein (0,008-2 mg L-1), 6MAM (0,005-2 mg mL-1) ve morfin (0.015-0,75mg mL-1) için metot tüm dağılım boyunca lineer bulunmuştur. Korelasyon katsayıları 0.997 ve 0.999 arasında değişmektedir.

Sonuç:

İnsan hakları talebindeki artışlar, özel sigortaların yaygınlaşması ile özellikle adli disiplinlerde olmak üzere toplam kalite prensiplerinin uygulamasını zorunlu hale getirmiştir. Güvenilirlik ve itibar mahkemelerce de önemli bulunmaktadır. Bu sebeple adli laboratuarlarda kullanılan metotların validasyonu ve devamlı iyileştirmesi oldukça önem kazanmaktadır.

Anahtar Kelimeler: Laboratuar, Kalite, Validasyon, Adli

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DETERMINATION AND METHOD VALIDATION OF HEROIN, MORPHINE, CODEINE AND 6-MONOACETYLMORPHINE BY GC-FID

Reliability and credibility are found very important also by the courts. For this reason validation and continual reclamation of methods that have been used in forensic laboratories is getting very important.

ABSTRACT

Objective:

This study describes the development and validation of a gas chromatography–flame ionization detection (GC–FID) method for the quantitative heroin (diacetylmorphine), morphine, codeine (3-methylmorphine) and 6-monoacetylmorphine (6 MAM) in narcotics.

Methods:

In our study, GC/FID Agilent 6890N (DB-5: 10,0m x 100 µm x 0,17 µm) with Chemstation software and 5% phenylmethylpolysiloxane non polar analytic colon (max 325 °C) was used for the analyses. Standards and reagents were Dichloromethane-isopropylalcohol mixture (1: 1), Methanol, HeroinHCl.H2O, CodeineHCl, MorphineHCl, 6-MonoacetylmorphineHCl, Paracetamol, caffeine and n-docosane which were used as reference standard and internal standard. Carrier gas was Helium. Injection temperature (T) was 280 C, injection volume was 1 µl. Repeatability study was evaluated using 4 operators. Reproducibility study was evaluated using 3 operators during 29 days.

Results:

The method is linear over the range (0.01-2.00mg mL-1) for heroin, (0,008-2 mg L-1) for codeine, (0,005-2 mg mL-1) for 6-monoacetylmorphine and (0.015-0,75mg mL-1) for morphine in the study. Correlation coefficients vary between 0.997 and 0.999 are detected.

Conclusion:

The increases in demanding of human rights, in getting prevalent of special insurances make it obligatory to perform total quality principles particularly for forensic disciplines. Reliability and credibility are also found very important by the courts. For this reason validation and continual reclamation of methods that have been used in forensic laboratories is getting very important.

Keywords: *laboratory, quality, validation, forensic*

INTRODUCTION:

Various processes in the plant may produce codeine, thebaine, and in some cases negligible amounts of hydromorphone, dihydromorphine, dihydrocodeine, tetrahydrothebaine, and hydrocodone (1).

Morphine is the most abundant alkaloid found in opium. It was discovered in 1804 by Friedrich Sertürner. In clinical medicine, morphine is regarded as the gold standard, or benchmark, of analgesics used to relieve severe or agonizing pain and suffering. Like other opioids, such as oxycodone (OxyContin, Percocet, Percodan), hydromorphone (Dilaudid, Palladone), and diacetylmorphine (heroin), morphine acts directly on the central nervous system (CNS) to relieve pain. Morphine can be taken orally, rectally, subcutaneously, intravenously, intrathecally or epidurally (2,3).

Unlike the opioids, morphine is an opiate and a natural product. Morphine has a high potential for addiction; tolerance and psychological dependence develop rapidly, although Physiological dependence may take several months to develop (4). Morphine and its major metabolites, morphine-3-glucuronide and morphine-6-glucuronide, may be quantitated in blood, plasma, or urine to monitor for abuse, confirm a diagnosis of poisoning or assist in a medicolegal death investigation (5).

Heroine is a semi-synthetic opioid synthesized from morphine, a derivative of the opium poppy. It is the 3,6-diacetyl ester of morphine. The white crystalline form is diacetylmorphine hydrochloride, however heroin freebase may also appear as a white powder. Frequent administration quickly leads to tolerance and dependence and has a very high potential for addiction. This is much quicker than other common opioids such as oxycodone and hydrocodone (6,7). When taken orally, heroin undergoes extensive first pass metabolism via deacetylation, making it a prodrug for the systemic delivery of morphine (8). When the drug is injected, however, it avoids this first-pass effect, very rapidly crossing the blood-brain barrier due to the presence of the acetyl groups, which render it much more lipid-soluble than morphine itself (9).

Codeine is an opiate used for its analgesic, antitussive, and antidiarrheal properties. Codeine is the second-most predominant alkaloid in opium. Codeine is considered as a prodrug. It is metabolised in vivo to the primary active compounds morphine and codeine-6-glucuronide (C6G) (10).

6-Monoacetylmorphine (6-MAM) or 6-Acetylmorphine with morphine and 3 acetylmorphine is one of three active metabolites of heroin. 6-monoacetylmorphine already has a free 3-hydroxy group and shares the high lipophilicity of heroin, so it penetrates the brain just as quickly and does not need to be deacetylated at the 3-position in order to be bioactivated; this makes 6-monoacetylmorphine somewhat more potent than heroine (11).

In modern analytical chemistry, the appropriateness of a chemical quantitative method employed for a particular analysis is often assessed through the cybernetic approach of method validation (12). The approach relies on the evaluation of some or all of the following factors: precision (repeatability, reproducibility), bias (spike recovery, deviation from certified reference value), linearity of measurements, sensitivity (limit of detection, limit of quantification), specificity (matrices interference, endogenous interference) and ruggedness test. Such a validation process provides the basic requisite for statistical characterization of the concerned method; however, further consideration of the relationship with metrological measurements like weighing, volume measuring, purity of standards used, etc. are not taken into account (13).

The aim of this paper was the quantification of active constituents (heroine, morphine, codeine and 6-monoacetylmorphine) and identification of adulterants such as caffeine, amitryptillyne, paracetamole present in the seized samples. Major alkaloid and adulterant data can also give

us important information about determination of seized samples origin. Especially South-East Asian heroine samples can be distinguished from those samples originating elsewhere with reasonable certainty by comparison of major alkaloid analyses (14).

This study describes the development and validation of a gas chromatography–flame ionization detection (GC–FID) method for the quantitative heroin (diacetylmorphine), morphine, codeine (3-methylmorphine) and 6-monoacetylmorphine (6 MAM) in narcotics.

MATERIALS AND METHODS:

Equipment: Chromatographic analysis was carried out on an Agilent 6890N Network gas chromatography system equipped with a flame ionization detector, an Agilent 7683 series autosampler, an Agilent chemstation (Balance : Mettler Toledo, Vortex: Nuvemix, Filter: Econofilter, PTFE, Pore size;0,45 µm., Diameter;25 mm).

Standards and Reagents: Dichloromethane-isopropylalcohol (1:1), Assay (GC) min. %99,7 was used as a solvent. Methanol was used as an irrigation solvent. HeroineHCl. H2O, codeineHCl, morphineHCl, 6-MonoacetylmorphineHCl, paracetamol, cafeine and n-docosane were used as reference standard and internal standard.

Method: DB-5 Column (10,0 m x 100 µm x 0,17 µm), 1 µl injection volume, 1/100 split ratio, oven temperature program; (initial: 150 oC, initial time: 0,00 min., ramps rate: 20 oC/min., final T: 310oC, hold time: 5min., total run time: 13 min., injection T: 280oC, carrier gas and flow: Helium 30.0 ml/min., detector gases and flow: Hidrojen 40,0 ml/min., air 450.0 ml/min.)

Samples (10-50mg) were dissolved in internal standard solution (0.5mg/ml n-docosane in dichloromethane:isopropanol,50:50,v/v) helped by vortex. After filteration, 1 µl solution injected to GC-FID.

In this study, validated analytical method was reliable and rapid. Samples of heroine, morphine, codeine and 6-monoacetylmorphine were analysed by GC-FID without extraction and derivatization.

All analytes were determined in same run. Tests for analytical method validation were carried out and measurement uncertainty calculated.

RESULTS:

The validation consisted of studies on linearity, specificity, accuracy, limit of detection and quantification, precision and robustness. The validation was performed according to the recommendations of EURACHEM guide (12).

The method was linear over the range (0.01-2.00mg mL-1) for heroine, (0.008-2 mg mL1) for codeine, (0.005-2 mg mL-1) for 6-monoacetylmorphine and (0.015-0,75mg mL-1) for morphine. Correlation coefficients vary between 0.997 and 0.999 were detected (Table 1).

Selectivity was assessed by interefances within the matrix. Parameters of chromatography was shown in table below. The method was determined very selective. Affect of interference of some adulterants such as caffeine, paracetamol present in the seized samples was analyzed for the specificity testing. From the RTs of the analytes, absence of interferences was seen. All substance's RTs are presented (Table 2).

Evaluation of the accuracy was carried out at two levels. The low level was referred to LOQs of the compound and any level above LOQs was referred of calibration curves of the compound in the study. At the level of LOQ relative error was calculated as -4,90% for heroine, -14,73% for morphine, -2,62% for codeine, and -3,40% for 6-MAM.

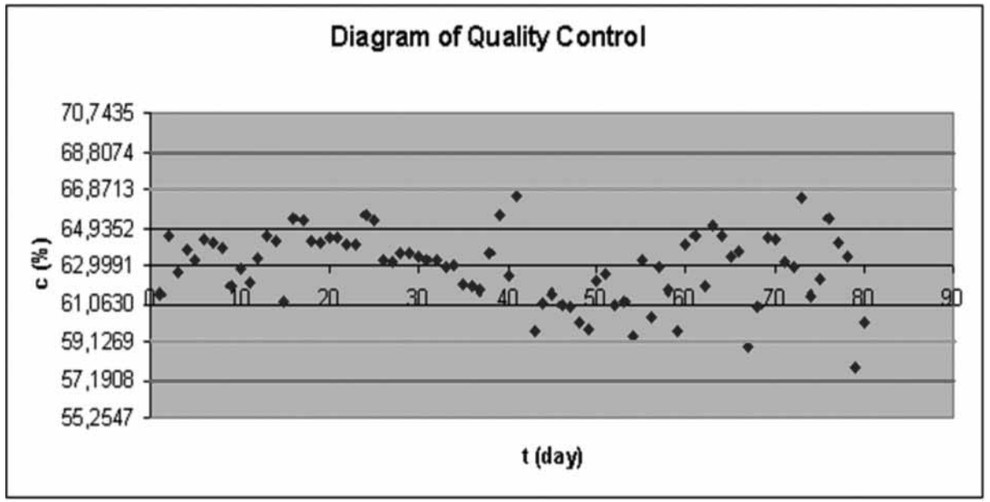
Limits of detection (LODs) vary between 5.30-14.20 µg/mL and limits of quantitation (LOQs) vary between 6,50-17,40 µg/mL (Table 3).

The precision of our method was determined by repeatability and reproducibility studies. Repeatability (intra-assay precision, RSDr, %) study was performed 10 times by 4 operators for heroine, morphine, codeine ve 6-MAM. Four operator were used an original sample including 60% heroine for heroine samples and were used mix standart for morphine, codeine ve 6-MAM samples in a day. Reproducibility (as between-day precision, RSDR, %) was performed during 29 days by 3 operator. RSD results were calculated and given (Table 4).

Robustness:

Results of test by changing of injection temparature and oven temparature and changing of time at 4°C confirmed the robustness of the method. The sample we used was analyzed for 6 times by 30 min., 1, 2, 3, 4, and 5 hours keeping at 4°C. There was no significant deviation in the results.

Quality Control (QC): Our method was used for quantitative analyzing of heroine (diacetylmorphine), morphine, codeine (3-methylmorphine) and 6-monoacetylmorphine (6 MAM) in narcotics by gas chromatography–flame ionization detection (GC–FID) (QC Graphic).



Graphic 1. Quality Control (QC)

Table 1. Calibrations curves and correlation coefficients (R2)

COMPOUND	CALIBRATION RANGE (MG/ML)	EQUATION	R^2
Heroin	0.010-2.000	y =1.0095x-0,0122	0.999
Morphine	0.015-0.750	y = 1.0307x-0,0129	0.997
Codeine	0.008-2 .000	y = 1.0024x-0,0033	0.998
6-MAM	0.005-2 .000	y = 1.0089x-0,0125	0.999

Table 2. *RTs of analytes and other compounds present in seized samples*

COMPOUND	RT	RT/RTISTD	WIDHT	RESOLUTION
Heroin	7,287	1,419	0,014	11,092
6-MAM	6,877	1,339	0,016	36,547
Codeine	6,282	1,224	0,015	44,100
Morphine	6,504	1,267	0,015	42,551
Docosane	5,133	1	0,015	38,350
Caffeine	3,395			
Papaverine	7,897			
Paracetamol	2,563			
Noscapine	9,501			
Thebaine	6,728			

Table 3. *Limits of detection and quantitation*

	HEROINE		CODEINE		MORPHINE		6-MAM	
	0,0100MG/ML	1,0000MG/ML	0,0080MG/ML	0,5000MG/ML	0,0150MG/ML	0,5000MG/ML	0,0050MG/ML	0,5000MG/ML
Avarage	0,0095	0,9650	0,0078	0,4406	0,0128	0,4659	0,0048	0,4457
SD	0,0004	0,0354	0,0006	0,0010	0,0005	0,0045	0,0002	0,0021
RSD	4,10	3,67	7,71	0,23	3,64	0,96	3,39	0,48
Error	-0,0005	-0,0350	-0,0002	-0,0594	-0,0022	-0,0341	-0,0002	0,0543
Relative-Error	-4,90	-3,50	-2,62	-11,89	-14,73	-6,82	-3,40	-10,85
LOD	10.70 µg/mL		9.60 µg/mL		14.20 µg/mL		5.30 µg/mL	
LOQ	13.40 µg/mL		13.80 µg/mL		17.40 µg/mL		6.50 µg/mL	

Table 4. *The within-day repeability and day-to-day reproducibility values of the parameters*

		HEROINE	6-MAM	MORPHINE	CODEINE
REPEATIBILITY	n	10	10	10	10
	RSDr	0,75	3,29	2,51	2,95
REPRODUCIBILITY	n	87	85	85	85
	RSDR	2,79	3,57	4,56	3,72

DISCUSSION AND CONCLUSION

The method for the determination of heroin (diacetylmorphine), morphine, codeine (3-methyl-morphine) and 6-monoacetyl-morphine (6 MAM) in narcotics was completely validated by using linearity, selectivity, accuracy, LOD and LOQ, precision and robustness parameters.

The linearity of the method were determined as (0.01-2.00mg mL-1) for heroin, (0.008-2 mg mL-1) for codeine, (0.005-2 mg mL-1) for 6-monoacetylmorphine and (0.015-0.75mg mL-1) for morphine. Correlation coefficients of linearity were seen between 0.997 and 0.999 (Table 1).

According to precision testing, there were no significant differences between the results of different operators within the 95% confidence level.

It was seen that Tebain and 6-MAM were interfering with each other according to selectivity (Table 2). We concluded that analysis of all samples including 6-MAM have to be confirmed by GC/MS.

The accuracy at the level of LOQ, relative error was calculated as -4,90% for heroin, -14,73% for morphine, -2,62% for codeine, and -3,40% for 6-MAM. All these relative error results were below 15%.

We successfully applied this method to the determination of diagram of quality control for heroin in narcotics by using interval ±2 and ±3 . By using this diagram and samples of quality control, quality of our laboratory analyses was guaranteed.

Judicial authorities and law enforcement agencies try to eradicate the illicit production and narcotic trafficking. Physical and chemical analysis of confiscated samples, with special attention for the identification and the quantification of active components it can be possible to assist these authorities by important information.

Also the increases in demanding of human rights, in getting prevalent of special insurances make it obligatory to perform total quality principles particularly for forensic disciplines. Reliability and credibility are found very important also by the courts. For this reason validation and continual reclamation of methods that have been used in forensic laboratories is getting very important.

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