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Simultaneous HPLC estimation of Amphetamine and Caffeine abuse drugs in Iraqi human addicts

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Abstract

The main aim of this research is to establish and validate a high performance liquid chromatography (HPLC) process for the separation and estimation of Amphetamine (AM) and Caffeine (CAF) in its illegal formula and in sera of addicts. This method is established on the HPLC separation of the two drugs on the ZORBAX ODS column (250×4.6×5µm particle size). The mobile phase contained 1% ortho-phosphoric acid 85% and 1%of diethyl amine 99%, acetonitrile and methanol ratio was 85:10:5 v/v/v. The flow rate is 1.2 mL.min^{-1}, buffer value pH of 2.5 via isocratic elution also UV detection at 210 nm. The retention times for the two drugs AM and CAF were obtained at 4.425 and 6.456 min, respectively. The calibration curves founded that the linear regression analysis data gave a good linear relationship for the concentration range 1 to 100 µg.mL^{-1} for AM and CAF. The values achieved for correlation coefficient, slope and intercept were 9999, 8104.2 and 5012 for AM and 0.9999, 9698.5 and 6342.9 for CAF, whereas the LOD and LOQ was 0.51, 1.64 µg.mL^{-1} for AM and 0.60, 1.32 µg.mL^{-1} for CAF

1. Introduction

Amphetamine (AM)-Methyl benzeneethanamine Fig.1 has a stimulant effect on the central nervous system (CNS) and it also a stimulant of the phenethylamine type. Amphetamine is a synthetic original drug that includes dextro-amphetamine (dexies), methamphetamine which called crystal or meth and it can be taken by smoking and injection [1, 2]. These drugs, which have the same effects are obtained as powder, tablets also capsule that can be used orally [1]. Caffeine (CAF) 1,3,7-trimethyl-1H-purine-2,6 (3H,7H)-dione, Fig.2 is a stimulant for the central nervous system and used medically to get back mental alertness when drowsiness occurs or unusual weakness. The 200 mg/day dose caused raised focus also a feeling better [3, 4 and 5]. The effective material in each drug was differed in their weight and structure; furthermore, their action and the primary illegal drugs are opiates, primarily heroin, cocaine, cannabis as well as amphetamine type stimulants like amphetamines and methamphetamine[6]. The world's most widely used drugs include nicotine, caffeine and alcohol, which are considered recreational drugs because they are used for pleasure and enjoyment rather than medicinal purposes. [7, 8] Common opiates such as morphine, cocaine and heroin are either fully synthetic or semi-synthetic alkaloids to imitative the action of a natural alkaloid opiate CAF has been determined using, LC, spectrophotometric and HPLC [9, 10, 11]. Abuse drugs can be detected by several analytical techniques such as ultraviolet, visible, fluorescence spectrophotometry [12, 13, 14], mass spectrophotometry (MS), TLC, NMR, gas and liquid chromatography with different detectors which apply to a broad range of compounds of interest to toxicologists, pharmaceutical, environmentalists and clinicians.[15,16,17]

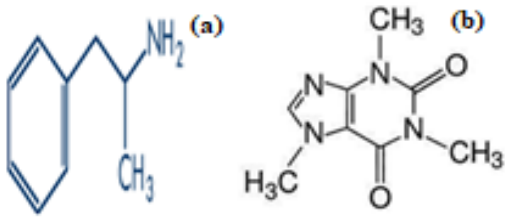

Figure1: **(a) chemical structure for AM, (b) chemical structure for CAF**

2. Chemicals and materials

2.1. Addicts biological life specimens

The application of this study were performed on a 60 addicts samples with an age ranged between 25-45 years selected from Ibn Rushd Hospital-Baghdad city, all were heavy smokers, which are divided into three groups, each group consisting of 30 addicts to 30 healthy as a control group. Addicts groups were classified to AM addicts (30 samples) and CAF addicts (30 samples). The blood samples were transferred into a gel tube left at room temperature for clotting, then centrifuged the samples at 3000 rpm for10 min in order transferred the serum samples to a sterile tube, they stored at -20°C until assayed time.

2.2. Buffer Preparation

The buffer solution contains water and 1% diethyl amine 99%, 1% ortho-phosphoric acid 85%, then adjusts pH to 2.5[18].

2.3 Mobile phase Preparation

A mixture of buffer and mobile phase is prepared: acetonitrile: methanol in the ratio 85: 10: 5 v/v/v. Then the mobile phase was filtered by using 0.45µm nylon filter.AM and CAF stock solutions Preparation The 100 $\mu g.mL^{-1}$ concentration of AM and CAF standard stock solution was immediately prepared via dissolving 10 mg of AM or 10 mg

of CAF into a 100 mL volumetric flask, then the volume was competed with methanol. The standard mixture which contained 10 µg.mL^{-1} of each drug was prepared also by mixing, then completing the volume with methanol.

2.4 Instrument and Chromatographic conditions

A Shimadzu, Kyoto, Japanese HPLC system is used, the analytical column was ZORBAX ODS (250×4.6×5µm particle size), Solvent reservoirs of 1000 mL capacity constructed from glass bottle were used. The HPLC system is equipped with a Shimadzu injection valve for manual sample injection with a 10 internal capacity sample loop.). The mobile phase containing Buffer (pH2.5): Acetonitrile: Methanol the ratio was 85: 10: 5 v/v/v., was filtered using a nylon filter membrane of 0.45µm pore size with isocratic elution mode at flow rate of 1.2 mL.min^{-1} and 10µL injection volume at column temperature of 25°C and using the UV-detector at wavelength 210 nm, the run time was about 8 min. In order to prevent the foreign particles to go through the column which may cause damage to the pump, also the active sites in the column might be pollute and decreases the column lifetime, a 0.45µm mesh pore size sterile syringe filters were used which give an important advantage that the analysis not requires the difficult and so complex step of pre-extraction of the drugs in the biological samples which may cause an errors in the result, also it needs several solvents and

2.5 Optimization of HPLC method

The two drugs AM and CAF were tested for chromatographic analysis by using different stationary phases, mobile phases with different pH and different flow rates. The retention time change, sensitivity and selectivity of each drug needed to change the ratio of mobile phase, pH, flow rate, and stationary phase. Methanol and water were used in diverse ratios but the peaks separation was incomplete notice, then acetonitrile and water were tested as a mobile phase in different ratios but low AM and CAF sensitivity were noted. The acetonitrile: methanol with buffer containing 1% phosphoric acid 85% and diethyl amine 99% at special pH were tested to get the best separation, pH 2.5 was showed the best results at different ratios of Acetonitrile: Methanol: buffer which give the best separation, symmetrical peaks, a good resolution, selectivity and sensitivity were by using the ratio 85:10:5 v/v/v of mobile phase at 1.2 mL.min^{-1} flow rate Figure. 4.

3. Discussion of the results

3.1.Validity of the HPLC method

Proving the validity of the HPLC optimization method was achieved with the following parameters

3.1.1. Precision and Accuracy

Asymmetrical peak without tailing also at constant tR was obtained via repeated three more times off on research analysis. The HPLC optimization method accuracy was achieved by estimating the values of percent relative error, RE % and precision was estimate by calculated the percent relative standard deviation RSD % values of each AM and CAF studied drug. Three replicate analyses for each AM and CAF drug at concentrations of 4, 8, 15 and 25 µg.mL^{-1} were tested which these concentrations were within the range of linearity for each drug.The results mention a suitable accuracy also precision of this developed method [19]. Standard solutions were measured and tested; calculate the practical drugs

concentration then comparing with the initial values added. The obtained results for the estimation of accuracy and the percentage recovery showed in Table 1. The analytical merit data was obtained by using linear regression first order equation for the calibration curve, which represents the relationship between analytic concentrations µg.mL^{-1} versus peak areas [20].

The calibration curve for a series of standard drugs solutions was prepared within the eight different concentrations a range of 1 to 100µg.mL^{-1} for AM and CAF, Figure 2, 3 .

Moreover, the estimated of the accuracy represented by percent relative standard deviation RSD% and precision represented by standard deviation S.D for this method was achieved by examining three replicate linearity data were recorded in Table 1, The analytical data for the determination of AM and CAF were showed in Table 2

Table 1. : Precision and accuracy of standard AM and CAF solutions via HPLC method

DRUG	initial Amount added (µg/ml)	Amount Found (µg/ml) Mean±SD	RE%	RSD%	Recovery %
AM	4	4.021±0.00510	+0.525 %	0.1268 %	100.5 %
	8	8.033±0.00281	+0.413 %	0.3498 %	100.4 %
	15	14.997±0.0066	-0.021 %	0.0440 %	99,98 %
	25	24.987±0.0112	-0.051 %	0.0448 %	99.94 %
CAF	4	4.013±0.0230	+0.325 %	0.573 %	100.3 %
	8	7.983±0.0071	-0.210 %	0.089 %	99.78 %
	15	15.066±0.009	+0.440 %	0.059 %	100.4 %
	25	24.957±0.045	-0.172 %	0.180 %	99.82 %

Table 2. Analytical data for the determination of AM and CAF

Parameters	AM	CAF
Dynamic range, µg/mL	1-100	1-100
Regressionequation	y = 8104.2x + 5012	y = 9698.5x + 6342.9
Correlation R^2	0.9975	0.9994
Correlation coefficient, r	0.9999	0.9999
Slope, b	8104.2	9698.5
Intercept, a	5012	6342.9
Standard deviation of Y-residual, Sy/x	829.3	935.7
limit of detection, LOD, µg.mL^{-1}	0.307	0.289
Limit of quantification, LOQ, µg.mL^{-1}	1.023	0.964

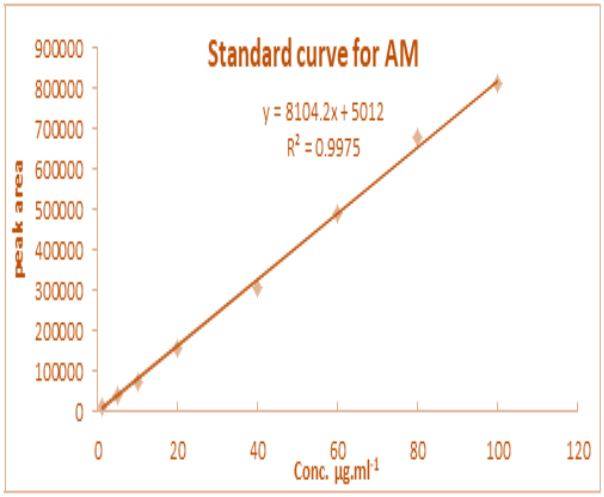

Figure 2. Calibration curve for AM using the developed HPLC method

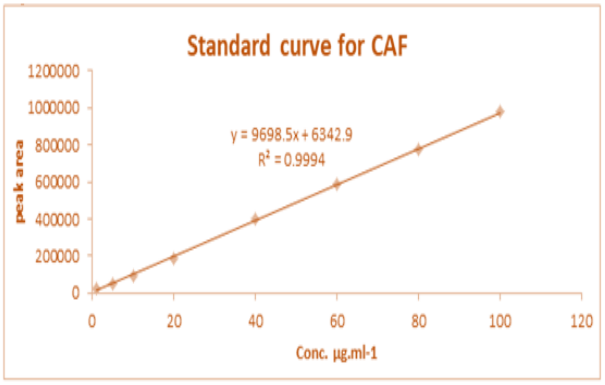

Figure 3. ***Calibration curve for CAF using the developed HPLC method***

3.3 Chromatographic functions:

A symmetrical peak without tailing also at constant t_R was obtained via repeated three more time off on research analysis. The chromatographic functions calculated [21]; resolution, Rs, 4.513 and separation or selectivity factor, α, 1.727 refers to acceptable values via completely separation, Table 3, Figure 4.

Table 3: ***Chromatographic parameters obtained via HPLC method***

Chromatographic parameters	AM	CAF
Retention time, tR min	4.425	6.456
Corrected retention time, tR' min	2.795	4.826
Number of theoretical plates, N	1958.06	1852.22
Effective number of theoretical plates, Neff	1388.80	1035.12
HETP, or H	0.1276	0.1349
Effective Height Equivalent to Theoretical Plates, Heff	0.1801	0.2415
Resolution, Rs	4.513	4.513
Separation factor, α	1.727	1.727

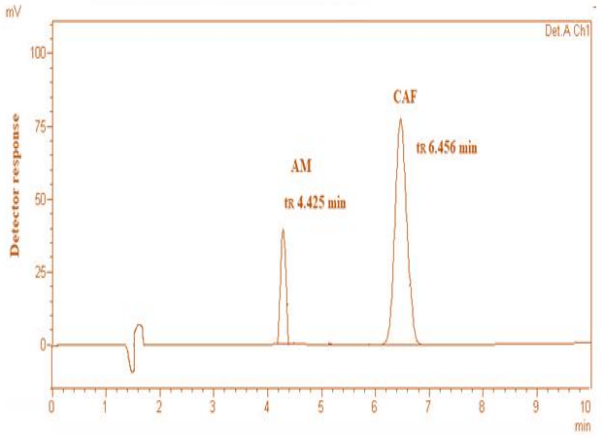

Figure 4: ***Chromatogram of AM and CAF drugs mixture at 50µg.mL^{-1}***

3.4. Addicts' samples analysis:

The developed HPLC method was applied for AM or CAF estimation via 60 human addicts in serum specimens collected from Ibn Rushd Hospital patients, so this method was effectively applied for identifying the addictive persons on their drugs and for follow up the addictive person who under treatment, this appeared significant peaks also concentrations for AM and CAF, as well as other peaks for unknown compounds found in these serum specimens, Figure 5.

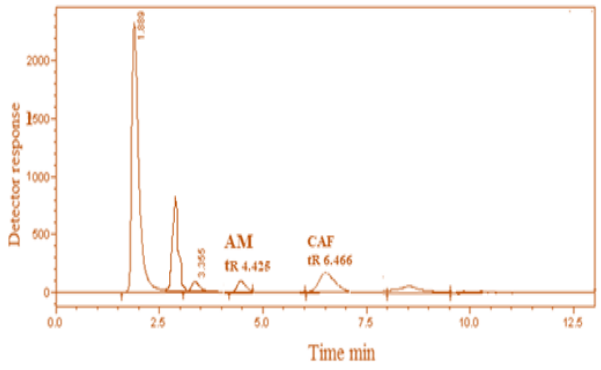

Figure 5: ***Chromatogram of AM and CAF drugs at addicts' serum specimens***

The concentration of drugs in serum specimens was calculated via the regression equation of the calibration curve of each drug which gives a highly significant increase for the level of each drug AM and CAF either in the serum specimens according to increasing the mean of the dose taken by addicts, Table 4

Table 4: Analysis of AM and CAF in sera of Iraqi addicts .

Dose of AM mg	Mean ±SD for conc of AM in serum	P value	Dose of CAF, mg	Mean ±SD for conc of CAF in serum	P value
250	28.56±4.265	0.0001	250	26.11±2.759	0.0001
500	86.50±5.981	0.0001	500	76.33±5.279	0.0001
1000	137.00±14.26	0.0001	1000	97.14±4.018	0.0001

CONCLUSION

This developed method gives good results for simultaneous amphetamine and caffeine estimation which combined together in any matrix either in the pharmaceutical dosage forms or in biological specimens. The concentration of AM and CAF can be estimated from serum specimens of addicts and determination qualitative and quantitative of these drugs from biological specimens obtained from addicted individuals taking different doses of these drugs by a new simultaneous HPLC method will be a benefit for developing new treatments for AM and CAF addiction and ensure compliance the addicts with medical treatment during clinical therapy. Finally, It is the potential to develop anther methods for other drugs by using the RP-HPLC method. This method with about 8 min runtime was good for the estimation of AM and CAF with a good resolution between each drug with high accuracy and precision with less than 2% RSD. The method is simple, accurate, precise, rapid and can readily use for routine work in any formulated form tablets, capsules and powder and in biological samples.

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References

[1]. Volkow. N; Boyle. M. (2018). Neuroscience of Addiction: Relevance to Prevention and Treatment. American Journal of Psychiatry, 175(8):729-740. doi: 10.1176/appi.ajp.2018.17101174

[2] . Farisco. M; Evers. K; Changeux. J,(2018), Drug Addiction: From Neuroscience to Ethics. Frontiers in Psychiatry, 9:595-609 . doi: 10.3389/fpsyt.2018.00595

[3] . Perry .CJ; Zbukvic. I; Kim .JH; Lawrence .AJ (2014). "Role of cues and contexts on drug-seeking behaviour". British Journal of Pharmacology. 171 (20): 4636–4672.

[4] . Carl Sherman , (2017) , The defining features of drug intoxication and addiction can be traced to disruptions in neuron-to neuron signaling. National Institute on Drug Abuse (NIDA) ,Advancing Addiction Science

[5] V. Pavlova and S. Petrovska-Jovanovic, Simultaneous Determination of Amphetamine, Methamphetamine and Caffeine in Seized Tablets by HPLC, ACTA CHROMATOGRAPHICA. No. 18. 2007: 157-167.

[6] .Ashok, A. H.; Mizuno, Y.; Volkow, N. D. & Howes, O. D,(2017), Association of Stimulant Use With Dopaminergic Alterations in Users of Cocaine,

Amphetamine, or Methamphetamine. JAMA Psychiatry, 74(5): 511-519. doi:10.1001/jamapsychiatry.2017.0135

[7]. Ti-Fei ; Ang , L; Xin S ; el al, (2016) , "Transgenerational Inheritance of Paternal Neurobehavioral Phenotypes: Stress, Addiction, Ageing and Metabolism" . Neuropsychology Review, Neuroscience ,Volume 53, Issue 9, pp 6367–6376

[8]. David M. Manuta ; Deborah F. Cate , (2018), False-positive drugs chemistry, Public Understanding of Chemistry,Manuta Chemical Consulting, Inc. Vol. 91 Number 1, pp 28-32

[9]. Dinis-Oliveira, R. J., Vieira, D. N., & Magalhães, T. (2016). Guidelines for Collection of Biological Samples for Clinical and Forensic Toxicological Analysis. Forensic Sciences Research.J , Vol 1(1): 42–51. doi:10.1080/20961790.2016.1271098

[10] . Nader Rifai. A; Rita Horvath Carl. T; Wittwer Andy Hoofnagle, (2018) , Principles and Applications of Clinical Mass Spectrometry , 1st eddition ,Sydney, Australia ,Science Direct, Elsevier Inc , Pages 33-65.

[11] . Bunaciu. A.A; Aboul-Enein .H.Y;Fleschin .S,(2010) ,Application of Fourier transform infrared spectrophotometry in pharmaceutical drugs analysis. Appl Spectrosc Rev. Vol;45(3):206–219.

[12] . Juliana Takitane; Rafael. M; Almeida; Tiago .F; et al , (2016), determination of Amphetamine, Amfepramone and Fenproporex in Urine Samples by HPLC-DAD: Application to a Population of Brazilian Truck Drivers , J. Braz. Chem. Soc., Vol. 27(3), 624-630.

[13] . Vahid Kiarostami; Mohamad-Reza Rouini; Razieh Mohammadian1 ; Hoda Lavasani; Mehri Ghazag ,(2014) , Inary Solvents Dispersive Liquid— Liquid Microextraction (BS-DLLME) Method for Determination of Tramadol in Urine Using High-Performance Liquid Chromatography , DARU Journal of Pharmaceutical Sciences . pp 22-25

[14] . Kiarash Fekri ; Mohammad-Reza Zarrindast ;Maryam Zahmatkesh ; Afsaneh Fard-Sanei ; Azadeh Nazari , (2018) , Comparison of High-Performance Liquid Chromatography and Thin Layer Chromatography for Identification of Amphetamine and Methamphetamine in Human Urine, Journal of Iranian Medical Council., Number 1 Volume 1 .

[15] . David M. Manuta ; Deborah F. Cate , (2018), False-positive drugs chemistry, Public Understanding of Chemistry , Manuta Chemical Consulting, Inc. Vol. 91 Number 1, pp 28-32

[16]. B. Sun, M. Li, F. Zhang, Y. Zhong, N. Kang, W. Lu, and J. Liu,(2010) , The performance of a fast testing system for illicit materials detection based on energy-dispersive X-ray diffraction technique, Microchem. J. 95(2); 293–297.

[17] . Wahhoodee S. M. and Al-Jassani M. J. A Forensic Study of Mycosis and its Relation with Amphetamine Addiction Tests, Journal of Global Pharma Technology, 2018,10(5), 153-156.

[18]. Matthias. Otto ,(2017) .Chemometrics Statistics and Computer Application in Analytical Chemistry, United States ,Wiley-VCH ,3rd ed ,pp 325-355.

[19] . Q. Zhao, C. F; Caiafa. D. P. Mandic; Z. C. Chao; Y. Nagasaka; N. Fujii, L. Zhang ; A. Cichocki, (2012) "Higher-Order Partial Least Squares (HOPLS): A Generalized Multi-Linear Regression Method", arXiv:1207.1230v1 [cs.AI], pp 1 -13.

[20] . HeiHwa Lee, Jong Feng Lee, Sin Yu Lin, Ping Ho Chen and BaiHium Chen, Simultaneous Determination of HFBADerivatized amphetamines and Ketamines in urine by GC-MS, J.Anal. Toxicol., 2011; 35(3): 162-169

[21] . James Miller ; Jane C Miller., Statistic and chemometric for analytical chemistry, Pearson Education Limited, Prentice Hall, 7th Ed.2018.