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Simultaneous determination of methaqaulone, saccharin, paracetamol, and phenacetin in illicit drug samples by hplc

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Abstract

Background: Saccharin, a low calorie artificial sweetener was found as a new diluent / adulterant present along with paracetamol and phenacetin in an illicit methaqualone sample.

Methods: All these components were simultaneously analyzed by the proposed reverse phase high performance liquid chromatography method using C_{18} column using acetonitrile: water (90:10 v/v) as mobile phase with a flow rate of 1mL / min.

Results: The percentages of saccharin, phenacetin, paracetamol and methaqualone in illicit drug sample were found to be 15.0, 45.6, 25.1 and 12.0 respectively. The method was validated for limit of detection, limit of quantification, linearity, accuracy, precision and reproducibility with the help of the exhibit and simulated samples.

Conclusions: The proposed method is simple, accurate and fast. It can be applied to the routine analysis of illicit methaqualone samples as well as for their impurity profiles for tracing the origin.

Keywords: Saccharin, Methaqualone, Diluents, Illicit drug samples, HPLC, Drug profiling

Background

The ever-growing problem of drug abuse is of great concern to the society. The drugs of abuse may be encountered in forensic practice in either pure form, diluted and/or adulterated forms. The reasons for the presence of many substances as impurities, diluents or adulterants in illicit drugs are often varied. Sometimes it may be unintentional because of the imperfect and bad manufacturing and laboratory practices. Most of the time these diluents and adulterants could be added as cutting agents to increase the bulk, dilute, complement or enhance the effects of the drugs and to mimic the taste of a genuine drug. The evidence suggests that illicit drugs are more commonly adulterated with either neutraceuticals such as sucrose, lactose, dextrose, mannitol and vitamins or pharmaceuticals that will mimic the taste of illicit drugs such as quinine, caffeine, paracetamol and aspirin or some innocuous substances such as talc, starch, chalk and magnesium stearate (Behrman 2008) etc. On one hand the identification as well as determination of a controlled substance is important for forensic science laboratories for prosecutorial purposes and on the other hand profiling of decomposition products, side reaction products, precursors, impurities, solvents, adulterants and diluents are of prime importance to trace the geographical origin of the illicit sample.

Paracetamol or acetaminophen, (Figure 1) is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post surgical pain and providing palliative care in advanced cancer patients. It was found as adulterant in illicit heroin, methaqualone, phenobarbitone, cocaine, methamphetamine (Atasoy et al. 1988; Battisti et al. 2006; Brunt et al. 2009).

Phenacetin (Figure 2) is an analgesic, once widely used but nowadays its use has been declined because of its adverse effects. It was reported as adulterant in illicit cocaine, methaqualone and heroin samples (Fucci 2004; Fucci & Giovanni 1998; Furst 2000).

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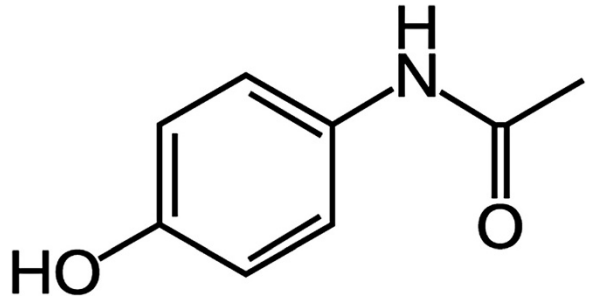

Figure 1 Paracetamol.

Methaqualone (Figure 3) is a sedative-hypnotic drug that is similar in effect to barbiturates, a general central nervous system depressant. It was widely used in the 1960s and 1970s as a hypnotic, for the treatment of insomnia, and as a sedative and muscle relaxant. It has also been used illegally as a recreational drug, commonly known as Mandrax. The drug was used during sexual activity because of heightened sensitivity and lowered inhibition coupled with relaxation and euphoria (Kacker & Zaheer 1951; Smyth et al. 1973).

Saccharin (1, 2-benzisothiazol-3(2H)-one-1, 1-dioxide), (Figure 4) is a non-glucose, low-calorie sugar substitute. It is found to be new cutting agent added in bulk or as diluent in illicit drugs because of its easy availability and low cost. Earlier in a study it was reported as diluent in illicit cocaine samples (Fucci & De Giovanni 1998). But in some recent cases saccharin was found in illicit methaqualone samples along with paracetamol and phenacetin.

Saccharin was determined individually in soft drinks, dietetic food samples (Filho & Nobrega 1994; Fo et al. 1993), determined simultaneously along with preservatives and flavoring agents in drinks (Ikai et al. 1988; Terada & Sakade 1985), also along with other non-nutritive sweeteners in dietetic samples (Chen et al. 1997; Biemer 1989; Zhu et al. 2005; Sastry et al. 1995; Valley et al. 2007). It was also determined individually in faces sample (Tibbels & Smith 1988). Limited references are available on the occurrence and determination of saccharin as diluent/adulterant in illicit drug samples

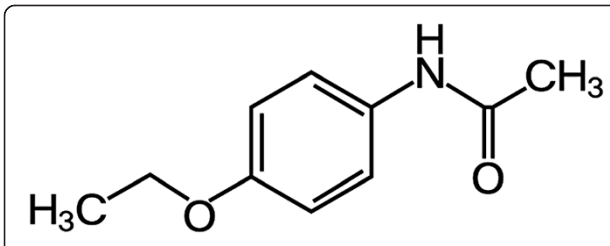

Figure 2 Phenacetin.

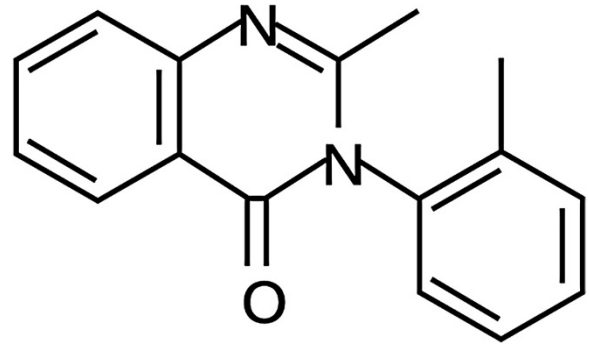

Figure 3 Methaqualone.

(Fucci & De Giovanni 1998). Most of the methods of analysis of drugs and other compounds were designed for their determination in routine pharmaceutical analysis and not for illicit drugs which might contain a variety of drugs and chemicals as diluents and adulterants, which are not present in official preparations. When the standard pharmaceutical procedures are applied to the illicit drugs they are fraught with interferences, difficulties in separation, identification and quantification. Therefore, there is always a need to develop new methods and procedures for the analysis of illicit drug samples where we can separate the adulterants, diluents and other impurities encountered in the forensic samples.

In this presentation a simple liquid chromatographic method has been described for the simultaneous determination of saccharin, paracetamol, phenacetin and

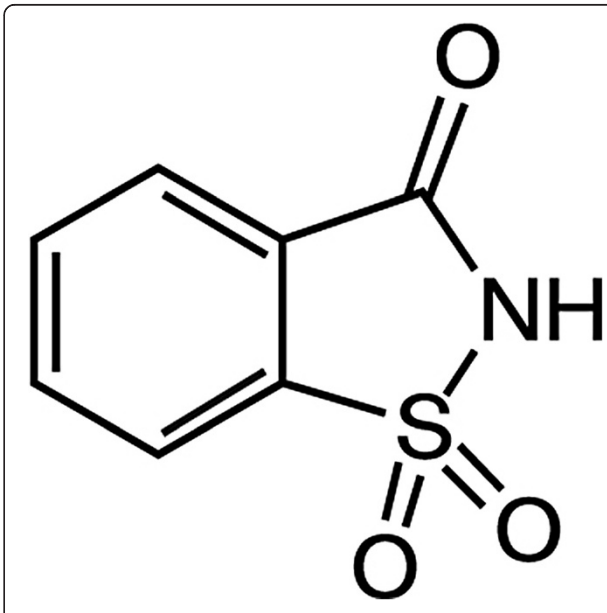

Figure 4 Saccharin.

methaqualone in an illicit methaqualone samples. To demonstrate the usefulness of this method samples were also analyzed by employing Clarke's HPLC methods (Anthony et al. 2003) for determination of paracetamol, phenacetin and methaqualone where as Tibbels and Smith method (Tibbels & Smith 1988) was used for analysis of saccharin.

Experimental

Chemicals and reagents

Saccharin was supllied by Kare Labs (India), paracetamol; phenacetin and methaqualone were purchased from Sigma-Aldrich (India). HPLC grade acetonitrile and water were purchased from Qualigens (India).

Apparatus

High Performance Liquid Chromatography (HPLC) System (Waters) consisting of a 600E controller pump, a 717 plus auto sampler, 2996 PDA detector and an inline-degasser. Millinium32 software for data processing and C_{18} (Waters, Spherisorb 5 um ODS2, 4.6 × 250 mm) analytical column was used for separation.

Standard preparation

A standard stock solution containing a mixture of saccharin, phenacetin, paracetamol, and methaqualone having a concentration of 2 mg/mL of each of these substances was prepared in the mobile phase. The stock solution was further diluted with mobile phase to give the five different concentrations (containing saccharin, paracetamol, phenacetin and methaqualone in the range of 0.2 μg/10 μL to 1.8 μg/10 μL, 0.4 μg/10 μL to 2 μg/10 μL, 0.4 μg/10 μL to 2 μg/10 μL and 0.2 μg/10 μL to 1 μg/10 μL, respectively). Five replicates of each of these five solutions were injected for plotting the calibration curve.

For limit of detection and limit of quantification a standard solution containing each of saccharin, phenacetin, paracetamol and methaqualone (1 mg/mL each) was diluted to give the different concentrations (0.05 μg/10 μL to 2 μg/10 μL). Recovery studies were carried out by standard addition method where three different concentrations of above said components were prepared with in the calibration range of corresponding components.

Sample preparation

A homogenized representative sample (10 mg) was transferred to a 10 mL volumetric flask and made up to the mark with mobile phase with intermittent shaking. Simulated samples were simultaneously prepared by mixing methaqualone, phenacetin, paracetamol and saccharin in different proportions. The simulated samples were analyzed by the proposed method for validation studies.

Standards and samples were ultrasonicated for 15 min and filtered through a Whatmann No.1 (Cellulose, Particle retention of 11μm) filter paper prior to injection into the HPLC system.

Chromatography

Chromatography was carried out at ambient temperature. The mobile phase consisted of acetonitrile and water (90:10). The flow-rate of the mobile phase was 1mL/min. 10 μL of each of the standard solutions of the four compounds were injected in the HPLC to prepare a calibration graph. Then ten micro liters of sample solution was injected and concentration of each component was determined through the calibration graphs of the respective standards.

Method validation

Preliminary validation of the method was performed by checking the linearity, precision, recovery, detection and quantification limits, and repeatability.

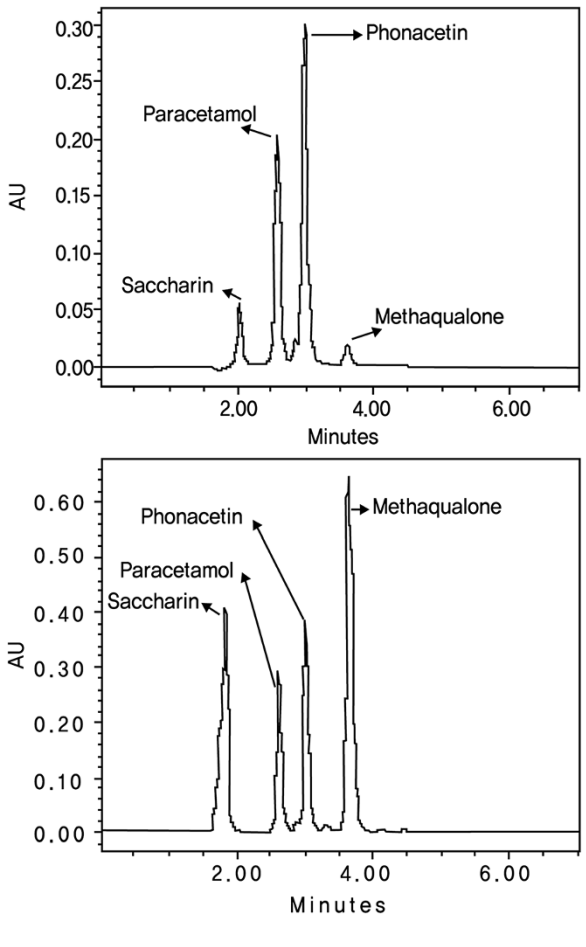

Figure 5 Showing the separation of paracetamol, phenacetin, methaqualone and saccharin in standard and illicit drug samples.

Limits of detection and determination

The detection and quantification limit were determined based on signal (S) to noise (N) ratio by injecting diluted solutions (made from stock solution (2 mg/mL)) into the HPLC system. Limit of detection (LOD) was calculated as S/N × 3 where as limit of quantification (LOQ) was calculated as S/N × 10.

Linearity

For linearity checking Stock solution (2 mg/mL) containing a mixture of saccharin, phenacetin, paracetamol and methaqualone was further diluted with mobile phase to give the final concentration of 0.1 μg/mL. And these solutions were injected into the HPLC system and the resultants peak areas of each component were recorded.

Precision

The precision of the method was evaluated on the basis of analyzing the three different concentrations of each component in the linearity range for repeating three times.

Recovery/accuracy

The accuracy of the method was expressed as the percentage recovery of each component. Recovery studies were carried out by standard addition method where three different concentrations of above said components were prepared with in the calibration range of corresponding components. And also recovery study was carried out by using simulated samples.

Repeatability

The consistency of the results for the same analytes samples were checked by repeating the experiment for 6 times per day (intraday) and consecutive for 3 days (interday). And standard deviation of the repeated recovery values was calculated.

Robustness

Robustness is a measure of a method's immunity to small but deliberate variations in the conditions used. Acetonitrile, water ratio (± 10%) and flow rate (± 10%) were deliberately changed and effects were monitored.

Table 1 Showing the data of limit of detection, limit of quantification and linearity range

Compound	Limit of detection (LOD)	Limit of quantification (LOQ)	Linearity range
Saccharin	15 ng	48 ng	200 ng – 1800 ng
Phenacetin	10 ng	33 ng	200 ng – 2000 ng
Paracetamol	6 ng	19 ng	100 ng – 2200 ng
Methaqualone	5 ng	16 ng	100 ng – 1400 ng

Table 2 Showing the regression equation, slope and intercept value of calibration curve obtained for different analytes

Analytes	Regression equation y = mx + c	Regression value (R^2)
Saccharin	Y = 1007.5x + 790110	0..9991
Phenacetin	Y = 1348.5x + 139111	0..9996
Paracetamol	Y = 2420.6x + 37789	0..9993
Methaqualone	Y = 5011.3x - 77962	0.9982

Results and discussion

In the analysis of illicit drugs like methaqualone, in addition to its identity and quantization, it is very important to give the complete profile of the sample with respect to the presence of active component, its decomposition products, side reaction products, impurities, adulterants and diluents so that this data can be compared and correlated with samples of known origin. Many methods are available for the analysis of individual components present in the samples. Analyzing individual components in different unknown samples is a time consuming and costly process. Under these circumstances methods which can determine the individual components simultaneously, accurately and quickly are desirable. The proposed method has been developed by keeping in view these factors. By the proposed method, which needs minimum sample preparation, it was possible to separate and determine saccharin, paracetamol, phenacetin and methaqualone simultaneously in illicit methaqualone samples.

The present HPLC method shows the well resolved peaks with a short analysis time of only 6 minutes. Saccharin, paracetamol, phenacetin and methaqualone were eluted at 2.0, 2.5, 3.0 and 3.6 minutes respectively

Table 3 Showing the recovery and precision data

Compound	Expected amount	Amount recovered in ng	% Recovery	±SD [% n=3]
Saccharin	600	600.16	100.02	4
	1000	1005.83	100.58	2.51
	1400	1408	100.57	3.51
Phenacetin	500	499.5	99.99	2.38
	1000	1015.33	101.53	2.73
	1500	1507.5	100.5	2.59
Paracetamol	500	507.16	101.43	2.27
	1000	1004.83	100.48	4.14
	1500	1500.66	100.04	4.66
Methaqualone	400	402.16	100.54	2.07
	600	598.33	99.72	3.28
	800	797.66	99.70	2.83

Table 4 Showing intraday and interday reproducibility data

Compound	Intraday recovery value (n=6)	± S.D	Interday recovery value (3 days)	± S.D
Saccharin	100.02, 101.31, 100.98, 99.99, 104.53, 101.54	1.66	100.02, 104.34, 102.54	2.16
Phenacetin	99.99, 99.87, 101.72, 103.64, 101.73, 100.11	1.47	98.99, 101.76, 102.70	1.92
Paracetamol	101.43, 102.70, 101.11, 100.68, 101.99, 99.76	1.02	101.43, 104.37, 104.01	1.60
Methaqualone	100.54, 100.96, 102.73, 101.13, 100.02, 99.99	1.05	100.54, 98.67, 103.54	2.45

(Figure 2) under the experimental conditions used. The samples were analyzed in the wavelength ranges from 210 nm to 350 nm by using PDA detector. For detection of saccharin, paracetamol, phenacetin and methaqualone an optimized wavelength of 238 nm was chosen. The mobile phase was optimized by varying mobile phase compositions and flow rate. It was found that mobile phase (acetonitrile: water, 90:10 v/v) with a flow rate of 1mL/min was found to be optimum for efficient resolution of the peaks (Figure 5).

The method was validated accordingly ICH guideline. The limit of detection (LOD), limit of quantification (LOQ), and linearity range and coefficient correlation data is presented in Table 1. The recovery of this method was found to be better than 99% (Table 2). And the standard deviation (+ SD) of this method was found to be in the range from 2 to 5 (Table 3). Intraday and interday studies also shows good reproducibility in respect of recovery (Table 4), and it was found to be precise and accurate. The method remained unaffected, by small but deliberate variations, in the LC flow rate (± 10%) and mobile phase ratio (± 10%). The quantification of the components was studied on simulated samples. The percentage of methaqualone, phenacetin, paracetamol and saccharin in a typical illicit drug sample as well as simulated samples in Table 5.

Conclusion

The proposed method is simple, accurate, reproducible and fast. It can determine the methaqualone, the adulterants/diluents including saccharin simultaneously. The present method can be routinely used for the analysis of these components in illicit methaqualone samples and it will be a valuable method for drug profiling.

Table 5 Percentage of amount found in different samples & comparative correlation of new method with reference methods

Sample	Compounds	% Amount expected	% Amount found by new method	% Amount found by reference methods	± SD (n=3)
Sample 1 (Unknown)	Methaqualone Phenacetin Paracetamol Saccharin	-	12.04	12.45	0.28
		-	45.60	45.09	0.36
		-	25.08	26.18	0.77
		-	15.01	14.31	0.49
Sample 2 (Mixed different proportion of four compounds)	Methaqualone Phenacetin Paracetamol Saccharin	25	26.15	24.68	1.03
		25	24.49	24.10	0.27
		25	23.98	25.35	0.96
		25	27.05	26.78	0.19
Sample 3 (Mixed different proportion of four compounds)	Methaqualone Phenacetin Paracetamol Saccharin	20	18.92	21.35	1.71
		20	21.05	21.69	0.45
		30	28.19	30.70	1.77
		30	32.11	29.85	1.59
Sample 4 (Mixed different proportion of four compounds)	Methaqualone Phenacetin Paracetamol Saccharin	30	31.98	30.51	1.03
		30	28.70	31.59	2.04
		20	19.25	18.75	0.35
		20	22.70	20.96	1.23
Sample 5 (Mixed different proportion of four compounds)	Methaqualone Phenacetin Paracetamol Saccharin	35	33.69	36.68	2.11
		30	28.18	31.32	2.22
		20	21.83	18.96	2.02
		15	13.76	14.13	0.26

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MI design the experiment, carried out the experiment, and contributed in framing the article. CJ assisted in the analysis using HPLC. PG collected the samples, assisted in the framing of experiment. SKS and TRB contributed in designing the experiment and framing the article. All authors read and approved the final manuscript.

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