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Identification of drugs using FTIR in a case of illegal abortion: A forensic study

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Abstract

Illegal or criminal abortion is defined as termination of pregnancy in the violation of law. In many cases, doctors perform abortion without the consent of parents or only mothers, which is an offence against mother and/or father. Abortifacient are being frequently used for the same purpose, misoprostol is one of the drug, whose role is to expel dead fetus and placenta by controlling uterine muscles. The aim of this study was to identify misoprostol, a pharmaceutical drug using ATR technique of FTIR by identifying peaks which lies in fingerprint region. Five major peaks, 3304, 2912, 1731, 1364 and 1016 cm^{-1}, were obtained, though as time lapses, peaks get deteriorate.

Keywords: Abortion; Misoprostol; FTIR; Acetonitrile; Pharmaceutical drug; Attenuated Total Reflection (ATR)

1. Introduction

Misoprostol, mol weight 382.53, $C_{22}H_{38}O_5$, comes under the prostaglandins group of medicine [1]. It is often used in the chemical abortion with another chemical mifepristone. IUPAC name of misoprostol is methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate. The role of misoprostol in the abortion is to ensure the expulsion of the dead fetus and placenta by producing uterine muscle contraction. Along with this, misoprostol is also used in the prevention of bleeding in postpartum hemorrhage, for induction of labor (it has oxytocin property) and to prevent stomach ulcers (it also has anti-ulcer property) [2,3].

Table 1 Different names of the drugs used in abortion

Sr. No.	Name of the drug	Analog ingredient of drug
1	Misoprostol	Prostaglandin E1
2	Dinoprostone	Prostaglandin E2
3	Ethacridine	Ethacridine lactate
4	Mifepristone	Mifepristone
5	Carboprost	Prostaglandin F2α
6	Hemabate/Carbopost Tromethamine	Prostaglandin F2α
7	Oxytocin	Oxytocin

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In the line of DNA profiling of the mother, child and father inevitable in the case of identity of the child and the determination of the biological father, a criminal case may also be affected under raises in the case of (i) if the child is aborted without the consent of mother and there by accusing the father and (ii) accusing the perpetrator who is responsible for the illegal abortion of the child without the consent of the mother and father. In the first case, the mother accuse the father as the first criminal who is responsible for aborting her child with the abetment of the medical officer, as a second criminal and on the other hand, in the second case, the doctor would be the first accused and the unknown person who is known to be as the medical officer is the second criminal. In these two cases the DNA profiling is not necessary for the identification of the child. But, it is necessary to identify the drugs which have been administrated orally or vaginally to the victim for the purpose of abortion. The suspected drugs which are being practiced for administering for the purpose of abortion are given in Table 1[4-9]. In this paper, the identification of misoprostol has been studied using FTIR.

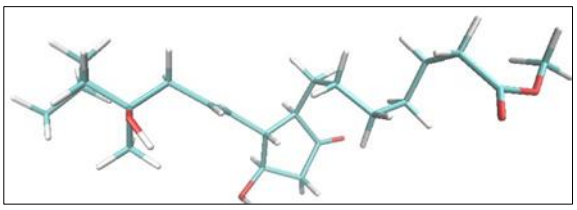

Figure 1 Structure of Misoprostol

Misoprostol is most soluble in ethanol and methanol. It is also soluble in DMSO, Dimethyl formamide and methyl acetate. Misoprostol maybe least soluble in water and sparingly soluble in acetonitrile. When misoprostol was crystallized using precipitating agent, polyethylene glycol, square prism shaped crystals were obtained [10]. The exposed medicine in the environment shows the decrease in the active ingredient dosage and increase in inactive degradation products [11] and when the stability of the said medicine is in question, to make it more stable the derivatives such as O-2,3,4,5,6-Pentafluorobenzylhydroxylamine hydrochloride (PFBHA) and 2,3,4,5,6-pentafluorobenzyl bromide (PFBB) are often used with 15(S)-15-methyl PGE_2 as internal standard and pentafluorobenzyl (PFB) and pentafluorobenzyl oxime (PFBO) are used for derivatization in GC-MS analysis [12-14]. Similarly, to stabilize other prostaglandins, methyl ester /methoxin / tert -butyldimethylsilyl (ME-MO-TBDMS) ether derivatives are used for PGE_2, PGD_2, 6-keto $PGF_{1\alpha}$ and methyl ester / tert-butyldimethylsilyl (ME-TBDMS) ether derivatives are used for 8-epi- $PGF_{2\alpha}$ and PGF2α [15].

A study was carried out to validate HPLC method for quantitation of misoprostol, run with mobile phase acetonitrile: water (85:15) in isocratic condition, in which Limit of Detection (LoD)and Limit of Quantitation (LoQ) of misoprostol was found to be 0.03 µl/ml and 0.10 µl/ml respectively [16]. In 2015, a RP-HPLC method was developed to estimate mifepristone and misoprostol simultaneously in their pharmaceutical dosage form. In the study, C8 column was utilized along with mixture of 0.02M potassium dihydrogen orthophosphate and 0.03M dipotassium hydrogen orthophosphate: Acetonitrile (60:40) as mobile phase. The Limit of Detection (LoD) for mifepristone and misoprostol were found to be 0.54 µg/ml and 0.69 µg/ml. the Limit of Quantitation for mifepristone and misoprostol were found to be 1.65 µg/ml and 2.1 µg/ml [17]. In 2019, the study for detection of illegal abortion using counterfeit medicine has been carried out using LC-MS/MS instrumentation [18]. In 2015, a case study was conducted in which mifepristone was detected in segments of hair at four different segments after the intake of medicine [19]. In 2003, a study was conducted regarding the pharmacokinetics of prostaglandin E1 analogue, misoprostol. The analysis of the assay was done using Gas Chromatography/ Negative ion chemical ionization mass spectrometry sing isotope dilution. According to the result, misoprostol acid had reached maximum concentration 20.9 pg/ml within one hour after oral administration [20].

Misoprostol metabolizes into misoprostol acid in body and its half-life is 20-40 minutes. The misoprostol and its acid could not be detected in the body fluid (e.g., Blood, urine, etc.) after 6 hours [21]. For the analysis of the misoprostol acid from blood, the blood sample should be centrifuge immediately and freezed in liquid nitrogen at -10̊ C to -20̊ C. Misoprostol acid should be analyzed within four hours, if left at room temperature. This gives approximately 8-10 hours for the analysis of the misoprostol acid from the time of administration of the medicine. However, if the medicine is recovered from the victim or its residue, then the misoprostol could be detected on the basis of the analytical analysis of the misoprostol. The coated capsule of misoprostol contains sodium starch glycolate, microcrystalline cellulose and hydrogenated castor oil. In 2014, a study was conducted in which misoprostol was analyzed using FTIR and showed peaks at 2930 cm^{-1}, 1735 cm^{-1}, 1381 cm^{-1}, and 1050 cm^{-1} [22].

The fingerprint region of FTIR for functional groups lies between 4000-400 cm^{-1}. In a molecule, vibration and rotation stretching occurring between two atoms originates the infrared spectra. The wavenumber depends upon the force constant and the reduced mass of the molecule. It is calculated as follows:

$$\bar{\upsilon} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

where, $\bar{\upsilon}$ is the wavenumber;
c is the speed of light;
K is the force constant;
and μ is the reduced mass.
Reduced mass is calculated as,

$$\mu = \frac{m1 \times m2}{m1 + m2} \times (Avogadro's\ number)$$

Force constant depends upon the bond strength and bond length between atoms and varies accordingly to the vibration or rotation stretching [23].

2. Material and Methods

The medicine was procured from the local reputed pharmacist for the purpose of the research in order to identify the medicine. Misoprostol, which gets converted into misoprostol acid, was checked for solubility by dissolving the same in various solvents such as water, ethanol, methanol, acetone, diethyl ether, benzene and phenol. The diluted medicine in various solvents was preserved in the vial, then vortex and rested in the sonicator for 15 minutes. It has been confirmed that the maximum percentage of the medicine was dissolved in ethanol comparatively higher than the other solvents. The medicine has also been found possessed with binders which is not dissolvable in the solvent. The misoprostol spectrum was taken using the Attenuated Total Reflection (ATR) method in the Fourier Transform Infrared Spectrometer (FTIR) for the presence and identification of the same instead of using the aborted content for the analysis.

2.1. FTIR

The instrument used is INFRA 3000-50 FTIR model of Analytical Technologies Limited Company, which has Mid IR range from 400 to 4000cm^{-1} and has Deuterated Triglycine Sulfate (DTGS) detector.

The medicine tablet was first crushed into fine powder and then the powder weighing approximately 0.05 gm was kept on the ATR stage and was scanned. After the completion of scan, peaks were obtained on the FTOS software and with peak selection, the specific peaks were obtained. The medicine was analyzed each time at the interval of one hour within the day with same method.

Table 2 Parameters of FTIR

Sr. No.	FTIR	Specifications
1	Model	INFRA 3000-50 FTIR
2	Company	Analytical Technologies Limited
3	Wavenumber range	4000 to 400 cm^{-1}, Mid IR
4	Scan Speed	16
5	Detector	DTGS detector
6	Beam Split Type	KBr
7	Resolution	(1)4WN

3. Results and discussion

The purpose of this model study is to determine the presence of the misoprostol through FTIR. In FTIR, the fingerprint region lies between 4000 – 400 cm^{-1}. Different chemical bond lies in different wavenumber region. The ranges of wavenumber according to different functional groups are given in Table 3.

Misoprostol mainly contains eight chemical bonds which includes -C=O(ester), -C=O (carbonyl bond), -C-OH (alcohol), -O-H (alcohol), -C-C- (alkane), -C=C- (alkene), -C-H (aliphatic) and $-CH_3$. The peaks obtained in this study are 3304 cm^{-1}[-O-H (alcohol)], 1737 cm^{-1} [-C=O(ester)], 1364 cm^{-1} [$-CH_3$] and 1016 cm^{-1}[-C-C- (alkane)]. Peaks obtained other than the desired are of the binders present in medicine.

It has also been observed that as time lapses, the active functional group degrade, resulting in the deterioration of the peaks obtained. The graph taken at interval of time shows that hydroxyl group peak deteriorates first followed by ester group. The first analysis of misoprostol is shown in figure-Spectrum 1 which has 4 peaks (1016, 1364, 1737 and 3304), after one hour, Spectrum 2 was obtained which showed three peaks of 1016, 1364 and 1737. Spectrum 3, 4 and 5 were taken at interval of one hour each and has only one peak of 1016. Spectrum 6 taken after one hour of Spectrum 5 shows no peaks. Table 4 shows the peaks obtained in FTIR Spectrum 1-6.

Table 3 Chemical Bonds present in misoprostol and their wavenumber range

Sr. No.	Chemical Bond present in Misoprostol	Wavenumber range (cm-1)	Observed wavelength (cm-1)
1	-C=O (Ester)	1700-1750	1737
2	-O-H (Alcohol)	3300-3700	3304
3	-C-C- (Alkane)	750-1100	1016
4	-CH3	1350-1480	1364

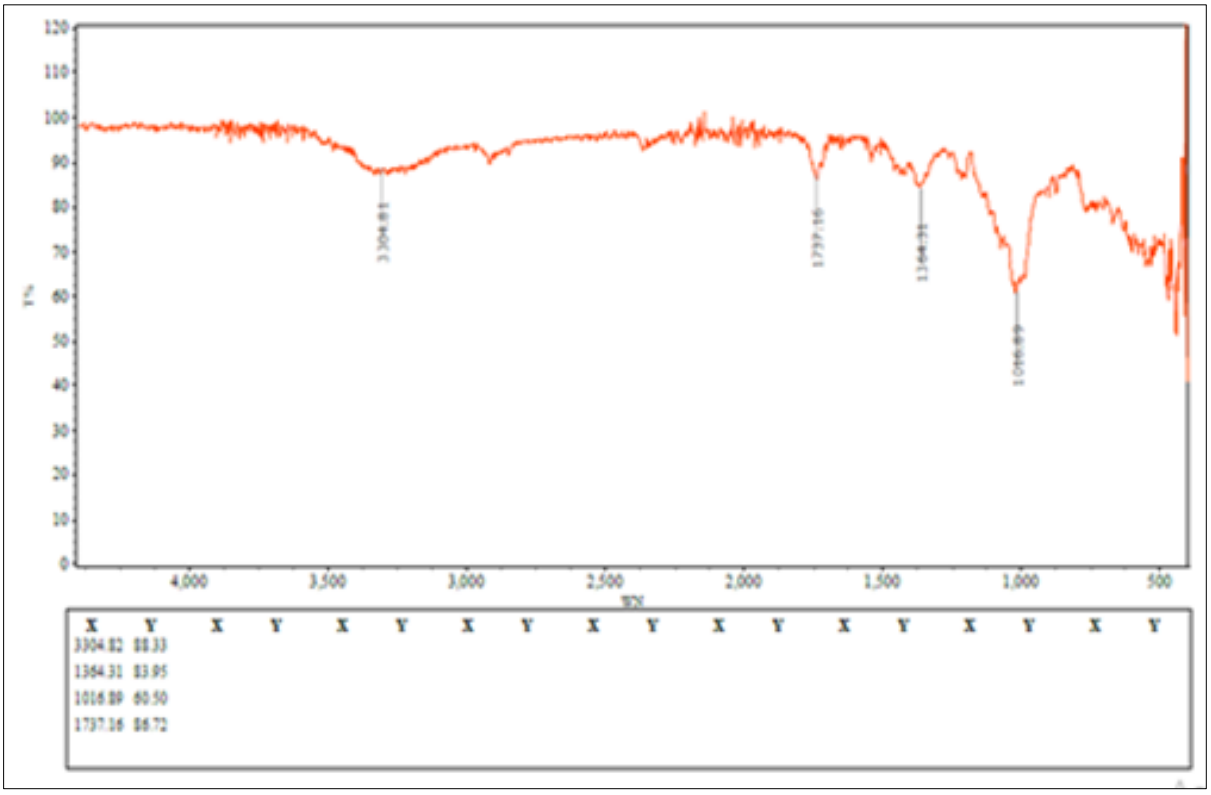

Figure 2 Spectrum 1

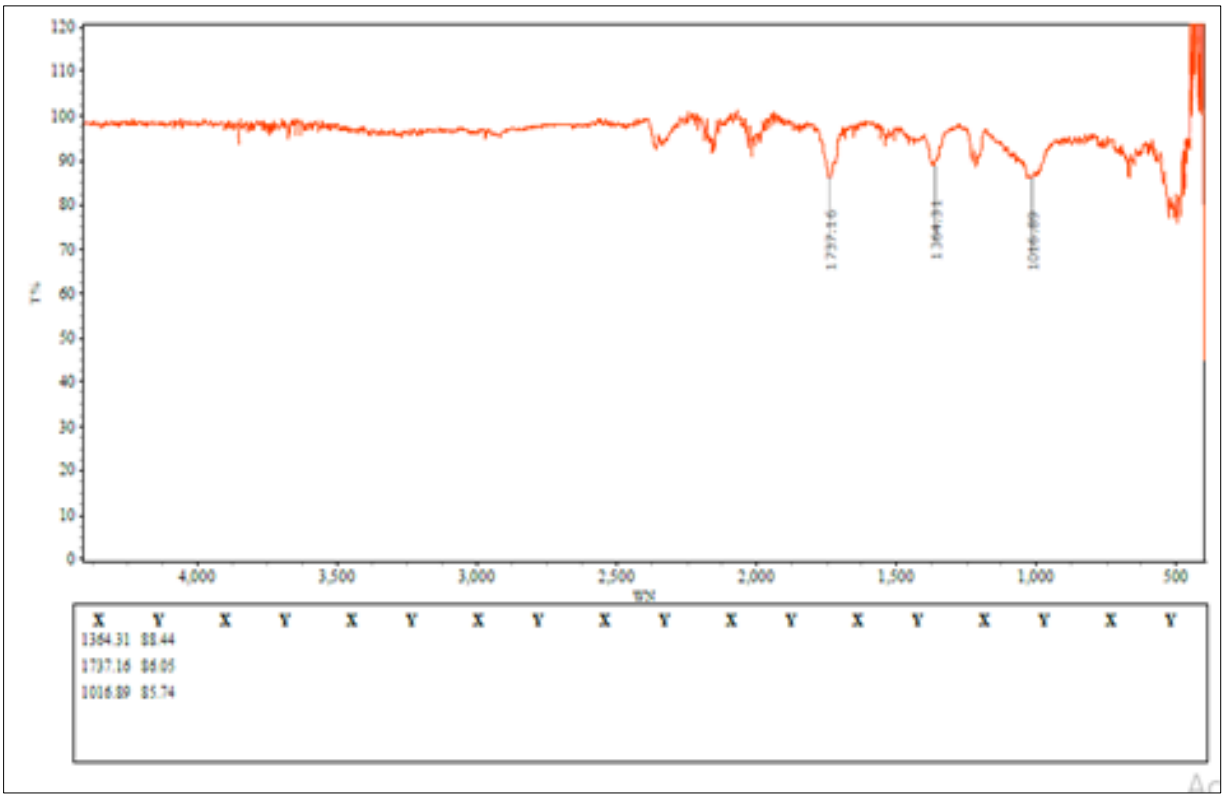

Figure 3 Spectrum 2

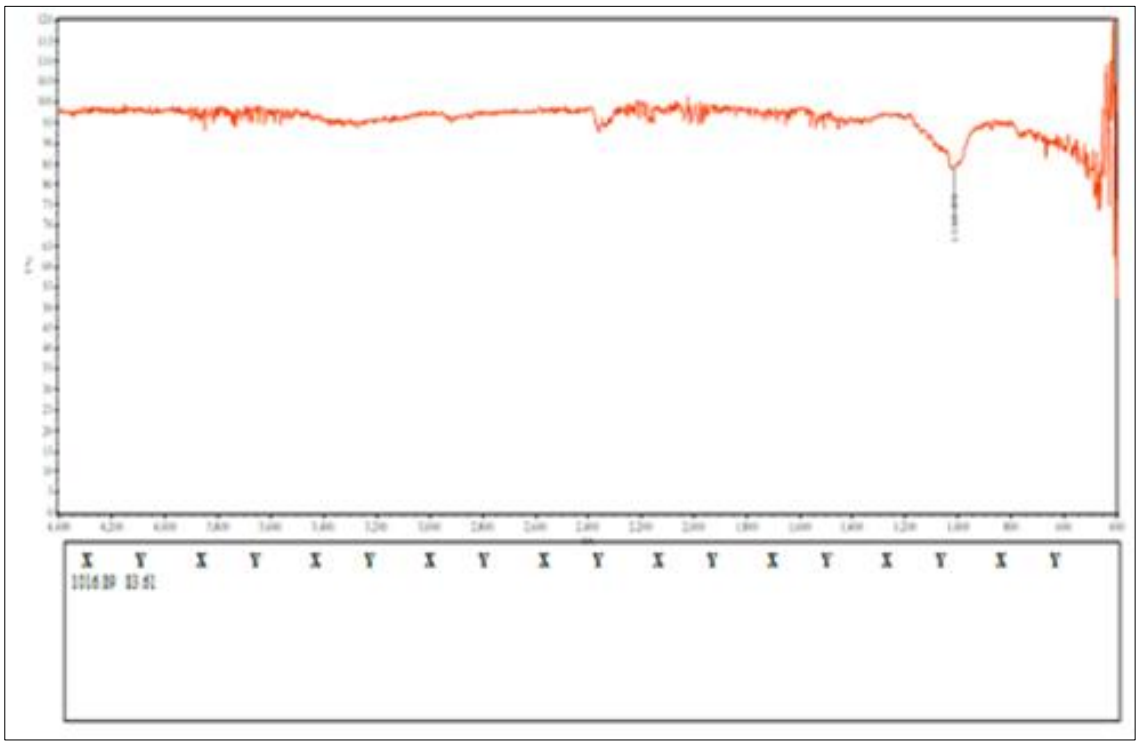

Figure 4 Spectrum 3

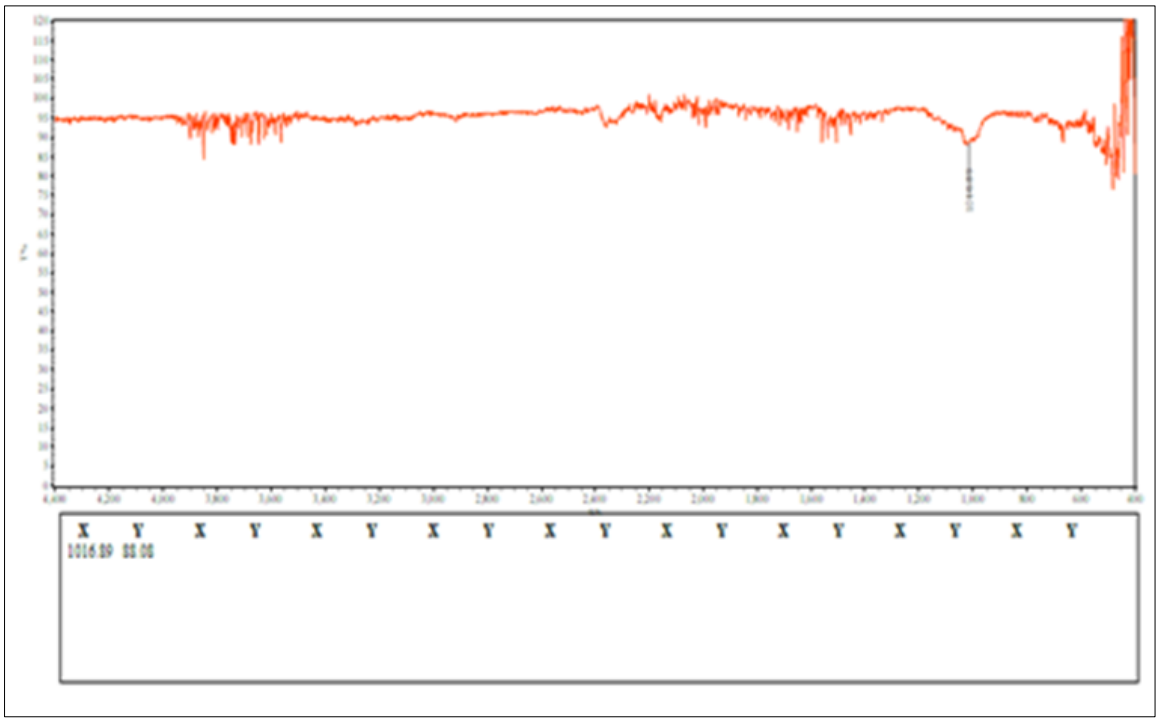

Figure 5 Spectrum 4

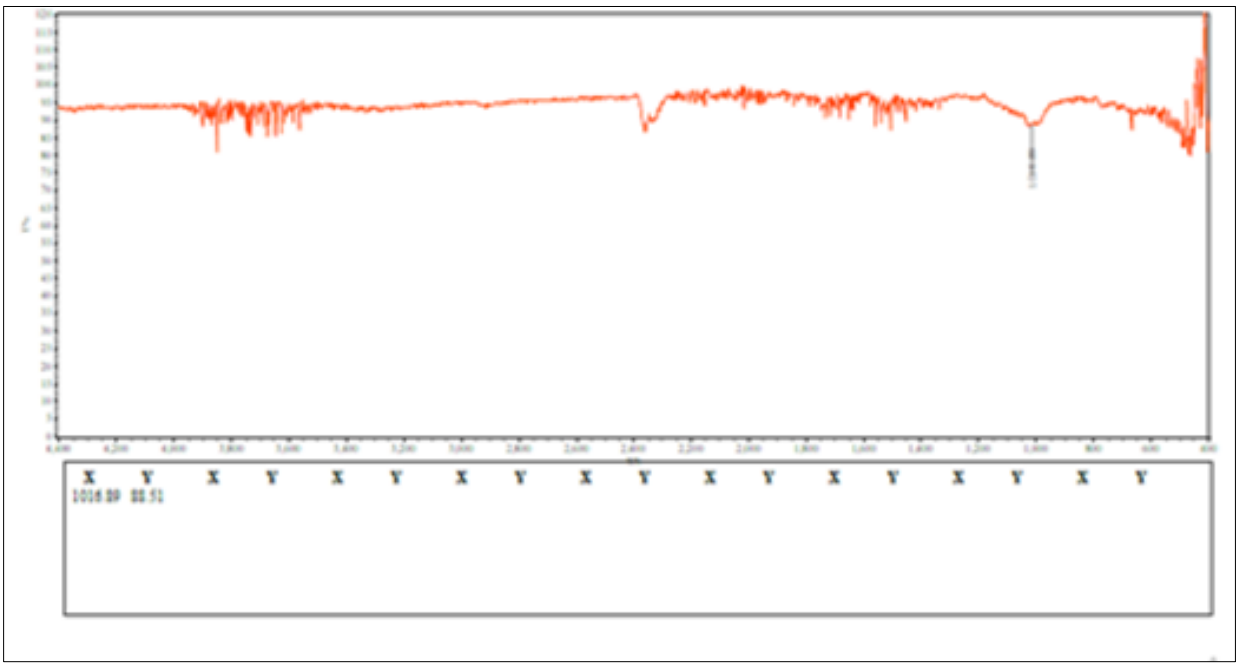

Figure 6 Spectrum 5

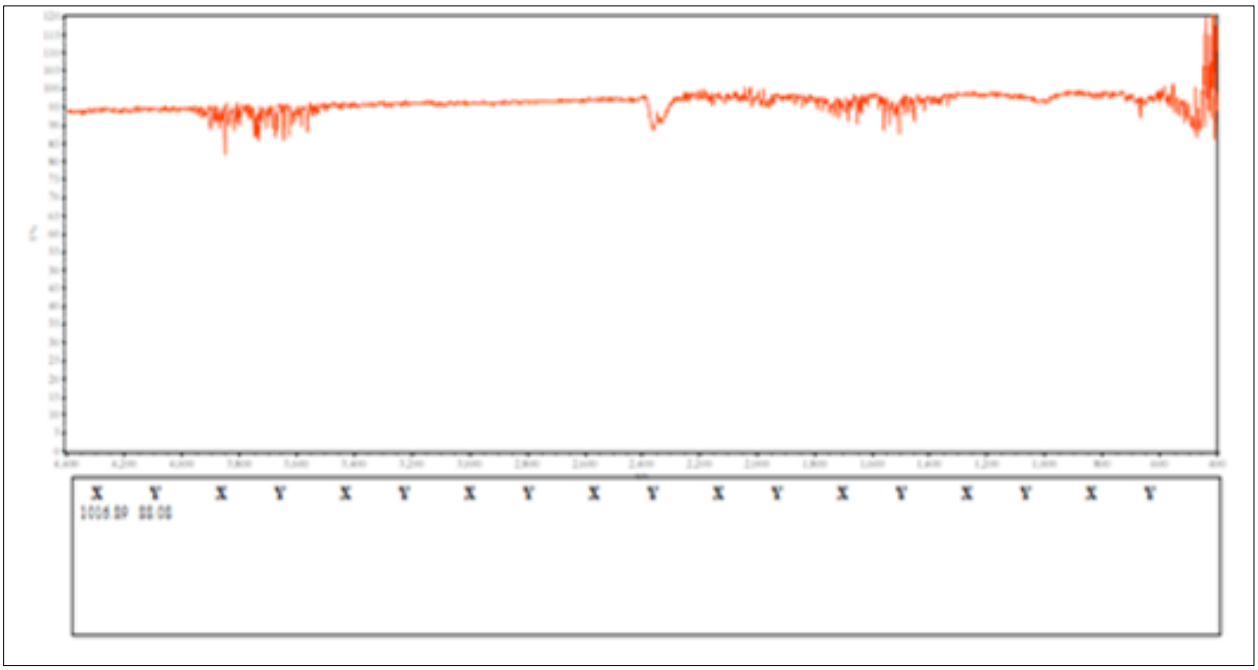

Figure 7 Spectrum 6

Table 4 Peak value in Spectrum 1-6 (each interval time of one hour)

Spectrum 1	Spectrum 2	Spectrum 3	Spectrum 4	Spectrum 5	Spectrum 6
1016	1016	1016	1016	1016	-
1364	1364	-	-	-	-
1737	1737	-	-	-	-
3304	-	-	-	-	-

4. Conclusion

With an increasing number of individuals opting for illegal abortion and when the case concerned referred to the forensic laboratories, it is inevitable and important to the forensic scientist to identify the drugs/medicine used for illegal abortion if the drugs/medicine is actually in practice in the abortion case. The analytical method used in this study helps in the identification of drug through its characteristic and functional groups in the spectrum. The presence decrease of peak height in the FTIR spectrum of misoprostol indicates the misoprostol gets degraded depends upon the time duration. The forensic analyst should aware that the examination for identification of drugs in such cases lapse of time will spoil the presence of drug which is deteriorating with time due to deteriorating the active functional group present in sample of the misoprostol.

Compliance with ethical standards

Acknowledgments

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Authors' contributions

Munusamy Baskar subject was initiated and supervised the whole work till the finalisation of the manuscript. Shivangi Gupta and Akanksha Singh analysed the sample, collection of references and manuscript preparation.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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