DRUGS IDENTIFICATION AND CHARACTERIZATION

As the compounds which have to be analyzed are mostly unknown, the first step, before quantification, is the screening and identification of compounds of interest. High selectivity and reliability are demanded, Maures (2005). Mamina *et al*.,(2002) stated that, in order to identify the drugs , it is possible to use chemical (color and precipitation reactions) or physiochemical methods (Thin layer chromatography TLC, ultraviolet spectroscopy UV)and proposed these method of screening of narcotic and intoxicating substances.

Different toxicological analytical techniques were applied to reveal the various factors, which may interfere with the characterization of the drugs (fentanyl and pentazocine). These techniques were color test, thin layer chromatography (TLC), ultraviolet spectroscopy (UV), gas chromatography (GC), infra red spectroscopy (IR) and Mass spectra (MS). The present work revealed the following results:

I) Spot test:-

Spot color tests are the first tests used, but they are not specific as they depend on variation of colors with the different drugs and reagents used. Since spot color tests usually screen for a class or a broad category of compounds, a positive result is usually considered to be presumptive only, Steinert and Coffman (1992). But its results combined with other identification techniques can be interpreted as definitive proof of the identification of different drugs. The present study revealed that marquis reagent gave orange color with fentanyl and red color with pentazocine.

Table (1) illustrates the different colors obtained by fentanyl and pentazocine using different chemical reagents, from which the following observations can be deduced.

Table (1): Colors produced by the studied drug using different chemical reagents

Reagent	Fentanyl	Pentazocine
Beam's	-ve	-ve
Chen-Kao	-ve	-ve
Cobalt nitrate/ ammonia	Pink	-ve
Cobalt thiocyanate	Blue	Blue
Concentrated sulfuric acid	-ve	-ve
Ferric chloride	-ve	Pale gray
FPN	-ve	-ve
Forrest's	Yellow	-ve
Frohde's	-ve	-ve
Furfural	-ve	-ve
Liebermann's	-ve	-ve
Mandolin's	-ve	-ve
Marquis	Orange	Red
PDA	-ve	-ve
Potassium permanganate	-ve	-ve
Potassium permanganate/ NaOH	-ve	-ve

Sodium hydroxide	**-ve**	**-ve**
Vitali- Morrine	**-ve**	**-ve**
Zwikker	**-ve**	**-ve**

No colors were obtained by fentanyl and pentazocine drugs with Beam's, Chen-Kao, concentrated sulfuric acid, FPN, furfural, Liebermann's, PDA, potassium permanganate, sodium hydroxide, Vitali- Morrine and Zwikker reagents. Thus these reagents are not suitable to identify the studied drugs.

On the other hand, only one reagent gave characteristic colors was found to be for fentanyl and pentazocine as follow:-

- Red color with Marquis reagent was produced with pentazocine and orange color was produced with fentanyl. These results could be explained as this reagent act on aromatic compounds which consist entirely of C, H and N, Clarke (2006).

Chromatography is one of the most efficient techniques in forensic chemical analysis. Its use in forensic expert examinations is of both applied and scientific importance. The present day forensic expert examination, the subject matter of which is the individual determination of a required object cannot be performed without reliable, highly sensitive, and selective chemical and physical techniques.

II)Thin layer chromatography (TLC):-

Various versions of chromatographic analysis are among these techniques thin layer chromatography (TLC) which is used both in target-oriented examinations for the identification of a particular substance and in the screening of materials of unknown nature. The methodological approaches to solving these problems are somewhat different from one another, this approach is currently used in the methodological recommendations approved by the Standing Committee for Narcotic Control of the Russian Federation (Fisteve *et al.*, 2004; and Bosch *et al.*, 2007).

Table (2) illustrate the colors obtained after spraying fentanyl and pentazocine with different coloring reagent.

Table (2): Colors produced by the studied drug using different chemical spraying reagents

Spraying color reagents	fentanyl	pentazocine
1- Examination under UV lamp using silica gel GF254	Blue	Blue
2- Mineral acids:		
a) Nitric acid 2%	-ve	-ve
b) Hydrochloric acid 2%	-ve	-ve
c) Sulphuric acid 2%	-ve	-ve
3- Reagent that are reduced with organic compounds:		
- Ferric chloride 5%	-ve	Pale gray
4- General reagents for basic Drugs:		
a) Dragendorff	Orange	Brown
b) Iodoplatinate	Violet	Brown
5- Aldehyde:		
a) Vanillin / sulfuric acid	-ve	-ve
b) Furfuraldehyde / sulfuric acid	-ve	-ve
6- Miscellaneous:		
a) PDA	-ve	Pink
b) FPN	-ve	-ve
c) Alcoholic sodium hydroxide	-ve	-ve
d) Potassium permanganate	-ve	-ve
e) Zwikker	-ve	-ve
f) Paladus chloride	-ve	-ve
g) Iodine vapour	Brown	Brown

As the color tests are not specific, a combination of thin layer chromatography (TLC) and color spot reactions have been used to identify various drugs, Clarke (2006). Visualization of spots of the drugs on the chromatoplates by different coloring reagents helps in locating the spots on the plates after elution by different eluting solvents. Burtis *et al.*, (1987) reported that spots of compounds in the TLC chromatograms are usually identified by their R_f values. However, Spratt (1977) demonstrated that the R_f value of a given drug may be vary according to humidity, temperature and size of the chamber used.

Fentanyl and pentazocine showed blue color under short UV lamp. Different coloring reagents were then sprayed on the chromatoplates, as direct coloring reagents. Spraying the chromatoplates by some mineral acids, reagents that are reduced with organic compounds, aldehyde reagents and some miscellaneous reagents as FPN, Zwikker reagent and alcoholic sodium hydroxide gave no color with the studied drugs.

Dragendorff reagent, iodine vapour and produced orange and brown colors respectively with fentanyl and pentazocine. As they give positive reaction with primary, secondary, tertiary and quaternary amines, Clarke

(2006). So these reagents could not be considered as specific spraying reagents for fentanyl and pentazocine.

It is of much interest to notice that iodoplatinate reagent may be considered as specific one to identify and differentiate the studied drug, since it produced a characteristic and specific color (violet with fentanyl and brown with pentazocine). Table (2).

This finding was found to agree with Clarke(2006) who reported that acidified iodoplatinate reagent acts on amino compounds and alkaloid bases to produce iodoplatinate complex.

Calculated R_f values for fentanyl and pentazocine: -

The main target aimed at the thin layer chromatographic studies of the drug was the separation and identification of the studied drug. The chromatographic behavior of fentanyl and pentazocine on silica gel GF_{254} is considered important for its identification. Different eluting solvents of moderate polarity were used in the present work with a considerable R_f values. Table (3).

Table (3) Calculated R_f values for fentanyl and pentazocine using different eluents

Eluents	Fentanyl	Pentazocine
(E1) Ethyl acetate	0.64	0.41
(E2) Ethyl acetate : Methanol: Ammonia (80:10: 10)	0.92	0.85
(E3) Chloroform: Acetone (4 : 1)	0.64	0.22
(E4)) Chloroform : Methanol (7 : 3)	0.92	0.69
(E5) Benzene : Ethanol (7 : 3)	0.83	0.70
(E6) Dichloromethane: Ether: Methanol: Water (77: 15: 8 : 1.2)	0.62	0.23
(E7) Hexane : Diethyl ether (4 : 1)	0.04	0.00
(E8)Cyclohexane: Acetone: Chloroform (70 : 25: 5)	0.58	0.34
(E9) Acetone	0.71	0.69
(E10) Acetone : Ammonia (80 : 15)	0.97	0.97

Fig. (1) Illustrate the chromatoplates of fentanyl and pentazocine using different eluents and sprayed with iodoplatinate reagent.

The present study demonstrated that fentanyl and pentazocine did not need eluting solvents of higher polarity. Different eluting solvents of moderate polarity were used in the present work with a considerable RF values. These are ethyl acetate (RF=0.64), ethyl acetate: methanol: ammonia80:10:10 (RF=0.92), chloroform: acetone 4:1 (RF=0.64), chloroform: methanol 7:3 (RF=0.92), benzene: ethanol 7:3(RF=0.83), cyclohexane: acetone: chloroform 70:25:5 (RF=0.58) and acetone: ammonia 80:15(RF=0.97) for fentanyl.

For pentazocine ethyl acetate: methanol: ammonia 80:10:10 (RF=0.85), benzene: ethanol 7:3 (RF= 0.70) and acetone: ammonia80:15 (RF=0.97).

These findings agreed with the Clarke (2006) who mentioned that different eluting solvents were convenient for thin layer chromatography detection of fentanyl and pentazocine. Also, the results obtained in this study agreed with (Dale *et al.,* 2000; Badwan *et al.,* 2001; Mikami *et al.*, 2002) as they used TLC for identification and determination of fentanyl and pentazocine using different elueting solvents of low and moderate polarities.

ii) Ultraviolet spectroscopy:

Ultraviolet spectrophotometric examination of drugs provides a simple and considerably reliable means of identification of pure drugs. Steinert and Coffman (1992) reported that the utility of spectrophotometry comes from the fact that each substance has its own absorption spectrum; therefore it can be used for its identification.

The present finding that each of both studied drugs has its own spectrum (250 nm for fentanyl and 277 nm for pentazocine), this agrees with the results of Clarke (2006).

Fig. (2,3) shows fentanyl and pentazocine UV spectrum, respectively.

IV)Gas chromatography:

The use of GC/MS for the identification and measurement of drugs of abuse is currently believed to be crucial to acceptance of evidence in legal proceeding because of its sensitivity and specificity. It is widely used to identify analytes, to determine concentrations, and to reveal the identity or differences in the compositions of trace components in test materials, Bosch *et al.,* (2007).

Several gas/mass chromatographic methods have been described for identification and determination of fentanyl and pentazocine (Kintz *et al.,* 1990; Watts and Caplan,1990; Schwarts *et al.,* 1994; Mautz *et al.,* 1994; Sachs *et al.*,1996; Fryirsa *et al.*,1997; Valaer *et al.,* 1997; Stout *et al.*,1998; Imamura *et al.,* 1999; Käferstein and Sticht,2000; Seno *et al.*,2000; Shou *et al.*, 2000; Dufersene *et al.,* 2001; Anderson and Mutto,2002; Ishii *et al.,* 2002; Maurer *et al.,* 2002a,b; Naidong *et al.,* 2002; Arinobu *et al.,* 2003; Kratzch *et al*., 2004; Maurer, 2004; Nadine *et al.*, 2004; Palvic *et al*.,2005; Chan *et al.,* 2007; Gupta *et al*., 2007; Teske *et al*., 2007; Moore *et al*., 2008; Moon and Chun 2008). Fig . (4,5)

V- Infrared spectroscopy:

Examination of fentanyl and pentazocine by using infrared spectrophotometry revealed that it may be identified through an equivalence of the absorption spectrum of the analyte with the characteristic infrared absorption spectrum (KBr pellet method).