التحديد النوعي لبعض مشتقات البنزوديازيبينات باستخدام كواشف نوعية

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الملخص

مع تزايد استخدام البنزوديازيبينات سواءً في العلاج أو عند استخدامها بشكل غير مشروع، أصبح من الضروري توعية العامة بمخاطر هذه الأدوية، حيث تشير الأبحاث إلى أن استخدام البنزوديازيبينات لفترات طويلة من الزمن قد يؤدي إلى الإدمان والاعتياد عليها. تعد فحوصات البقعة الكيميائية الجنائية إحدى الأدوات المستحدثة التي يستخدمها رجال الشرطة والأجهزة الأمنية للكشف عن المواد المخدرة والمساهمة في القبض على المتورطين في تهريبها وترويجها. تعتمد هذه الاختبارات على التفاعل الكيميائي الذي يحدث ما بين المادة المشبوهة والمواد الكيميائية الخاصة (الكواشف)، مما يؤدي إلى التعرف السريع على نوعية المادة المخدرة. مع ذلك يجب أن تؤخذ في الحسبان بعض التحديات التي تواجه هذه الاختبارات. يتطلب استخدام هذه التقانة خبرة ومعرفة كيميائية متخصصة. كما يمكن أيضًا أن تكون العينات المشتبه فيها ملوثة أو تالفة، مما يجعل من الصعب تحديد المواد المخدرة بدقة. ينبغي استخدام هذه الاختبارات كجزء من الطرائق الأخرى لتحليل المواد الدوائية المخدرة لضمان الدقة والموثوقية. يتضمن بحثنا هذا دراسة وإيجاد كواشف نوعية يمكن من خلالها الكشف السريع عن هذه المواد المخدرة عن طريق تشكيل ألوان مميزة ما بين المواد الدوائية المدروسة والكواشف المقترحة.

الكلمات المفتاحية: بنزوديازيبينات، اختبار البقعة الكيميائية، الاختبارات اللونية.

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Qualitative Determination of some Benzodiazepine Derivatives Using Specific Reagents

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ABSTRACT

The increasing use of benzodiazepines, both in treatment and when used illegally, it has become necessary to educate the public about the dangers of these drugs, as research indicates that the use of benzodiazepines for long periods of time can lead to addiction and dependence. Forensic chemical spot tests are one of the new tools used by police and security services to detect narcotics and help arrest those involved in smuggling and promoting them. These tests rely on the chemical reaction that occurs between the suspicious substance and special chemicals (reagents), which leads to the rapid identification of the type of narcotic substance. However, some challenges facing these tests must be taken into account. The use of this technology requires specialized chemical expertise and knowledge. Suspect samples can also be contaminated or damaged, making it difficult to accurately identify narcotics. These tests should be used as part of other methods for analyzing narcotic drugs to ensure accuracy and reliability. Our research includes studying and finding specific reagents through which these narcotic drugs can be rapidly detected by forming distinctive colors between the studied drugs and the proposed reagents.

KEYWORDS: Benzodiazepines, Chemical spot test, Colorimetric tests**.**

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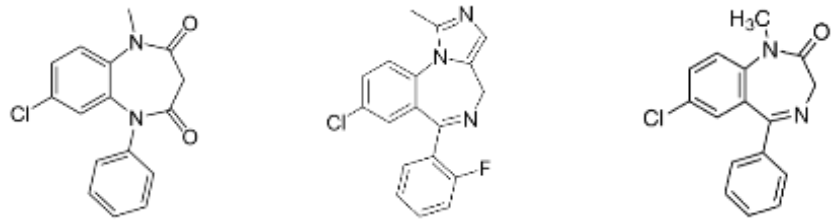
INTRODUCTION

Benzodiazepines (BZD_S) were introduced as therapeutic drugs in the early 1960 and are nowadays widely used for treating psychiatric and neurological conditions, including insomnia, anxiety disorders, alcohol dependence, and epilepsy. Some are also used as pre-anesthetic and intra-operative medications. The (BZD_S) share a common mechanism of action and produce similar pharmacological effects [1, 2]. The abuse or misuse of (BZD_S) is one of the potential serious social problems worldwide [3]. Since 2007, a variety of novel benzodiazepines has entered the international illicit drugs market. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is currently monitoring the prevalence and related harms of 33 novel benzodiazepines [4].

Diazepam, Midazolam, and Clobazam are among the most prominent derivatives of the benzodiazepine group [5-7], Its basic chemical structure consists of two benzene rings bonded to a diazepine ring, as shown in figure (1).



Clobazam(c) **Midazolam (b)** **Diazepam(a)**

Figure (1): (a) Structural formula of Diazepam (DZP).
(b) Structural formula of Midazolam (MDZ).
(c) Structural formula of Clobazam (CLZ).

Crime scene investigators (CSIs) often encounter unknown powders, capsules, tablets, and liquids at crime scenes, many of which are controlled substances. Because most drugs are white powders, however, visual determination of the chemical identity is difficult [8].

Since their introduction decades ago, colorimetric chemical detection tests or spot tests are also referred to as virtual drug tests based on their initial identification of unknown substances and help in identifying drug substances at the scene with rapid color change within 1-2 minutes or even within a few seconds. Colorimetric analyzes by testing a specific color of drug substances are a quick and simple way to determine the presence of drugs. This test can be performed in a short time and has a clear, easy-to-understand result. In addition, the

cost of this test is usually low compared to other drug screening tests. However, this test has many drawbacks and challenges. Keep in mind that these tests may be inaccurate and you may need to perform additional tests using other methods to confirm the result. In addition, the result may be affected by external factors such as contamination or other chemical reactions in the sample. In general, color analyzes by testing a specific color of narcotic substances are an important tool in combating drugs and determining their presence. This test provides quick and easy results and may be a valuable tool in controlling drug abuse and maintaining public health and safety [8-14].

It is very useful to find new detecting reagents helping forensic chemistry spot tests for the identification of drug substances which are a powerful and effective tool in achieving drug control goals. It also helps guide investigations, provides crucial evidence, and contributes to reducing the spread of drugs and protecting communities from their harmful effects. However, we must consider it as a complementary tool to integrated laboratory analyzes and work to develop and improve this technology to achieve maximum efficiency and accuracy in drug control [15-18]. A list of the ten most commonly used reagents for colorimetric testing of target drug classes is provided for reference in table 1 [19].

Zimmermann's reagent is used to detect some types of benzodiazepines, as it gives a reddish-purple color with diazepam. There is also the Vitaii-Morin reaction, which is used to detect some types of benzodiazepines, as it gives an orange to yellow color with benzodiazepine derivatives [20].

It is worth noting that benzodiazepine derivatives have been quantitatively determined by several methods, including: High-performance liquid chromatography (HPLC), Liquid chromatography with tandem mass spectrometry (LC-MS-MS), Spectrophotometric [21-23].

Table (1): Color test reagent compositions and targeted drugs for common color spot tests used in illicit drug detection.

Test reagent	Composition	Targeted drugs
Chen-Kao	- Acetic acid solution (1% v/v). - Copper sulfate solution (1%w/v). - Sodium hydroxide solution (8% w/v).	Ephedrine (and nor ephedrine)
Dille Koppanyi	- 0.1 g cobalt (II) acetate tetra hydrate in 100 mL methanol with 0.2 mL glacial acetic acid. - 5 mL isopropylamine in 95 mL methanol.	Barbiturates
Ehrlich	- 1 g p-di methyl amino benzaldehyde in 10 mL methanol, then add 10 mL conc. o-phosphoric acid.	Ergot alkaloids and LSD
Mandelin	- 1 g ammonium vanadate in 1.5 mL water, diluted to 100 mL with conc. sulfuric acid.	Amphetamines and anti-depressants
Marquis	- 1 mL formaldehyde (40% v/v) in 100 mL conc. sulfuric acid.	Broad spectrum test: mostly opium alkaloids and amphetamines
Scott	- Cobalt thiocyanate (2% w/v) diluted 1:1 with glycerin. - Conc. hydrochloric acid. - Chloroform.	Cocaine(and methadone)
Zimmerman	-2,4-dinitrobenzene (1% w/v) in methanol. -Potassium hydroxide (15% w/v).	Benzodiazepine derivatives and synthetic cathinones
Zwikker	- 0.5 g copper (II) sulfate pentahydrate in 100 mL distilled water. - 5 mL pyridine to 95 mL chloroform.	Barbiturates
Mecke	- 1 g selenious acid in 100 mL conc. sulfuric acid.	Opium alkaloids
Liebermann	- 1 g sodium/potassium nitrite in 10 mL conc. sulfuric acid.	Phenols and substituted benzene rings

Experimental:

1- Materials and Methods:

1-1- Tools:

Eppendorf tube, micropipette, Petri dishes, Whatman filter paper 40 µm, volumetric flasks with multiple capacities, and Sartorius balance sensitivity 10^{-5} g.

1-2- Materials:

Copper sulfate pentahydrate $CuSO_4.5H_2O$ 99 % from Himedia (India), Nickel sulfate hexahydrate from $NiSO_4.6H_2O$ 98.5 % Hopkin & Williams (Britain), Cobalt chloride hexahydrate $CoCl_2.6H_2O$ 99 % Hopkin & Williams (Britain), Potassium thiocyanate KSCN 98.5 %

Hopkin & Williams (Britain), Methanol 99 % from Merck (Germany), Hydrochloric acid HCl 37 % Sham lab (Syria), and Glacial Acetic Acid 98.5 % from Sham lab (Syria).

1-3- Primary pharmaceutical materials:

Diazepam (DZP) $C_{16}H_{13}ClN_2O$ from (India) 100%, Midazolam (MDZ) $C_{18}H_{13}ClFN_3$ from (India) 100%, Clobazam (CLZ) $C_{16}H_{13}ClN_2O_2$ from (India) 99.1 %, Valsartan (VAL) $C_{24}H_{29}N_5O_3$ from (India), Hydrochlorothiazide (HTZ) $C_7H_8ClN_3O_4S_2$ from (China), Ibuprofen (IB) $C_{13}H_{18}O_2$ from (India) and Diclofenac potassium (DP) $C_{14}H_{11}Cl_2NO_2K$ from (India)

2- Preparation of solutions:

2-1- Preparation of pharmaceutical materials for benzodiazepine derivatives in medium methanol:

Pharmaceutical solutions were prepared individually from Diazepam, Midazolam, and Clobazam by dissolving 50 mg of raw material in methanol volumetric flask 10 mL and completed the volume with methanol to give concentration equivalent to 5000 μg/mL.

2-2- Preparation of qualitative reagents:

2-2-1- Tetra chloro cobaltic II acid complex $H_2[Co(Cl)_4]$ reagent (R_1):

Tetra chloro cobaltic II acid complex $H_2[Co(Cl)_4]$ was prepared by dissolving 1.80 g of cobalt chloride hexahydrate in distilled water, then we added 12 mL of concentrated hydrochloric acid in volumetric flask 25 mL and completing to volume with distilled water.

2-2-2- Tetra acetato cobaltic II acid complex $H_2[Co(AC)_4]$ reagent (R_2):

Tetra acetato cobaltic II acid complex $H_2[Co(AC)_4]$ was prepared by dissolving 1.80 g of cobalt chloride hexahydrate in distilled water, then we added 10 mL of glacial acetic acid in volumetric flask 25 mL and completing to volume with distilled water.

2-2-3- Tetra chloro cupric II acid complex $H_2[Cu(Cl)_4]$ reagent (R_3):

Tetra chloro cupric II acid complex $H_2[Cu(Cl)_4]$ was prepared by dissolving 2.23 g of copper sulfate pentahydrate in distilled water, then we added 15 mL of concentrated hydrochloric acid in volumetric flask 25 mL and completing to volume with distilled water.

2-2-4- Tetra chloro nickelic II acid complex $H_2[Ni(Cl)_4]$ reagent (R_4):

Tetra chloro nickelic II acid complex $H_2[Ni(Cl)_4]$ was prepared by dissolving 2.00 g of Nickel sulfate hexahydrate in distilled water,

then we added 12 mL of concentrated hydrochloric acid in volumetric flask 25 mL and completing to volume with distilled water.

2-2-5- Tetra iodo cobaltic II acid complex $H_2[Co(I)_4]$ reagent (R_5):

Tetra iodo cobaltic II acid complex $H_2[Co(I)_4]$ was prepared by dissolving 1.80 g of cobalt chloride hexahydrate in distilled water, then we added 0.1 g iodine dissolved in methanol in volumetric flask 25 mL and completing to volume with distilled water.

2-2-6- Tetra thiocyanato cobaltic II acid complex $H_2[Co(SCN)_4]$ (R_6):

Tetra thiocyanato cobaltic II acid complex $H_2[Co(SCN_4)]$ was prepared by dissolving 1.44 g of cobalt chloride hexahydrate in distilled water, then we added 2 g of potassium thiocyanate and we added 8 mL of concentrated hydrochloric acid in volumetric flask 20 mL and completing to volume with distilled water.

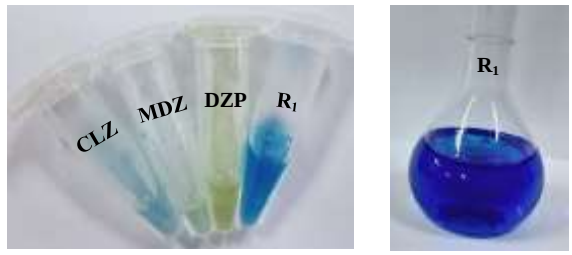
3- Result and discussion:

3-1- Chemical spot test procedures:

Three pharmaceutical compounds subject to drug control from derivatives of the benzodiazepine group were tested using several specific reagents in two ways. The first depends on placing a small amount of the derivatives of the benzodiazepine in its solid form in Petri dishes, adding drops of the reagent and monitoring the result. The second depends on using a liquid substance from these derivatives dissolved in ethanol as shown in figures (2-7). Table (2) shows the results of tests of these reagents in their individual form on drug-controlled substances for benzodiazepine derivatives and table (3) shows the results of tests of the reagents to non-narcotic drug substances.

Clobazam **Midazolam** **Diazepam** **Reagent(R_1)**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_1) in solid form.

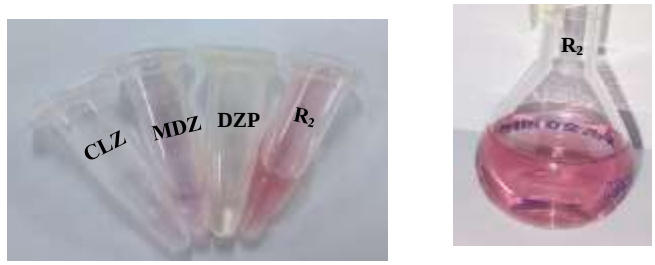


b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_1) in liquid form.

Figure (2-a,b): Results of applying the first specific reagent (R_1) to pharmaceutical derivatives CLZ, MDZ, DZP in their solid and liquid forms.

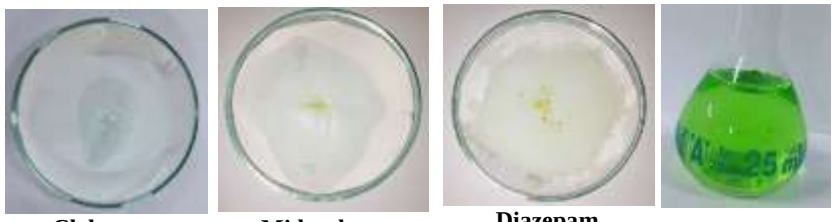
Clobazam **Midazolam** **Diazepam** **Reagent(R_2)**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_2) in solid form.



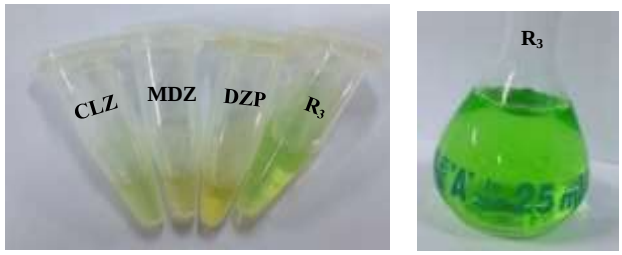
b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_2) in liquid form

Figure (3-a,b): Results of applying the second specific reagent (R_2) to pharmaceutical derivatives in their solid and liquid forms.



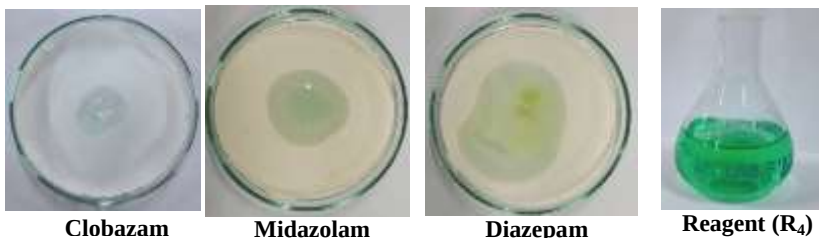
Clobazam **Midazolam** **Diazepam**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_3) in solid form.



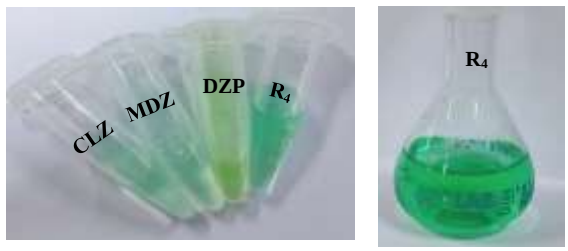
b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_3) in liquid form

Figure (4-a,b): Results of applying the third specific reagent (R_3) to pharmaceutical derivatives in their solid and liquid forms.



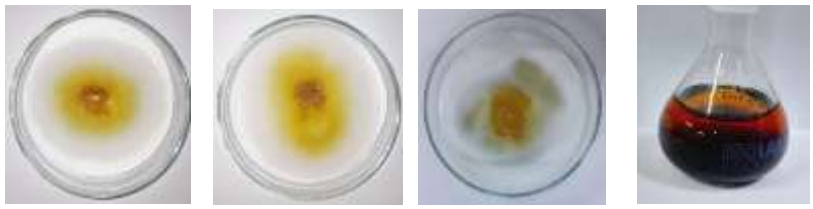
Clobazam **Midazolam** **Diazepam** **Reagent (R_4)**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_4) in solid form.



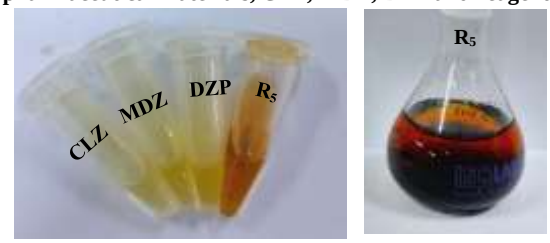
b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_4) in liquid form

Figure (5-a,b): Results of applying the fourth specific reagent (R_4) to pharmaceutical derivatives in their solid and liquid forms.



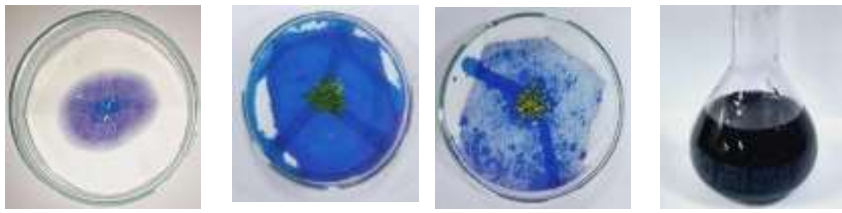
Clobazam **Midazolam** **Diazepam** **Reagent (R_5)**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent(R_5) in solid form.



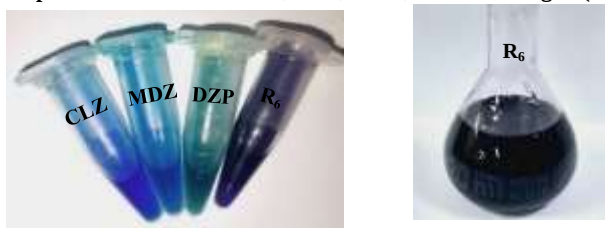
b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent (R_5) in liquid form

Figure (6-a,b): Results of applying the fivth specific reagent (R_5) to pharmaceutical derivatives in their solid and liquid forms.



Clobazam **Midazolam** **Diazepam** **Reagent (R_6)**

a: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent(R_6) in solid form



b: Narcotic pharmaceutical materials, CLZ, MDZ, DZP and reagent(R_6) in liquid form.

Figure (7-a,b): Results of applying the sixth specific reagent (R_6) to pharmaceutical derivatives in their solid and liquid forms.

Table (2): List of narcotic pharmaceutical compounds and their interaction with innovative specific reagents solid form.

$H_2[Co(Cl)_4]$ (R_1) blue			$H_2[Co(AC)_4]$ (R_2) pink		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Diazepam**	**Green Color**	**1**	**Diazepam**	**Yellowish brown Color**
2	**Midazolam**	**Blue Color**	**2**	**Midazolam**	**Purple color**
3	**Clobazam**	**No Color**	**3**	**Clobazam**	**No Color**
$H_2[Cu(Cl)_4]$ (R_3) yellowish green			$H_2[Ni(Cl)_4]$ (R_4) green		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Diazepam**	**Yellow color**	**1**	**Diazepam**	**Yellowish green**
2	**Midazolam**	**Yellow color**	**2**	**Midazolam**	**No Color**
3	**Clobazam**	**No Color**	**3**	**Clobazam**	**No Color**
$H_2[Co(I)_4]$ (R_5) brown			$H_2[Co(SCN_4)]$ (R_6) Blue-violet		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Diazepam**	**Reddish brown**	**1**	**Diazepam**	**yellowish green**
2	**Midazolam**	**Reddish brown**	**2**	**Midazolam**	**Green Color**
3	**Clobazam**	**Reddish brown**	**3**	**Clobazam**	**Blue Color**

Table (3): List of non-narcotic pharmaceutical compounds and their interaction with innovative specific reagents solid form.

$H_2[Co(Cl)_4]$ (R_1) blue			$H_2[Co(AC)_4]$ (R_2) pink		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Valsartan**	**No Color**	**1**	**Valsartan**	**No Color**
2	**Hydrochlorothiazide**	**No Color**	**2**	**Hydrochlorothiazide**	**No Color**
3	**Ibuprofen**	**No Color**	**3**	**Ibuprofen**	**No Color**
4	**Diclofenac potassium**	**No Color**	**4**	**Diclofenac potassium**	**No Color**
$H_2[Cu(Cl)_4]$ (R_3) yellowish green			$H_2[Ni(Cl)_4]$ (R_4) green		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Valsartan**	**No Color**	**1**	**Valsartan**	**No Color**
2	**Hydrochlorothiazide**	**No Color**	**2**	**Hydrochlorothiazide**	**No Color**
3	**Ibuprofen**	**No Color**	**3**	**Ibuprofen**	**No Color**
4	**Diclofenac potassium**	**No Color**	**4**	**Diclofenac potassium**	**No Color**
$H_2[Co(I)_4]$ (R_5) brown			$H_2[Co(SCN_4)]$ (R_6) Blue-violet		
No	**Drug Name**	**Color Observation**	**No**	**Drug Name**	**Color Observation**
1	**Valsartan**	**No Color**	**1**	**Valsartan**	**No Color**
2	**Hydrochlorothiazide**	**No Color**	**2**	**Hydrochlorothiazide**	**No Color**
3	**Ibuprofen**	**No Color**	**3**	**Ibuprofen**	**No Color**
4	**Diclofenac potassium**	**No Color**	**4**	**Diclofenac potassium**	**No Color**

We conclude from table (3) that non-narcotic drug substances are not affected by innovative specific reagents, which indicates the effectiveness and selectivity of reagents for derivatives of the benzodiazepine group, as shown in table (2).

3-2- Specificity:

The specific reagent tetra chloro nickelic II acid complex $H_2[Ni(Cl)_4]$ (R_4) is a selective detector for diazepam in both solid and liquid forms, as it gives a negative result with the other two derivatives. In addition, the result was positive for midazolam with all reagents except the reagent (R_4): $H_2[Ni(Cl)_4]$, the two reagents tetra iodo cobaltic II acid complex $H_2[Co(I)_4]$ (R_5) and tetra thiocyanato

cobaltic II acid complex $H_2[Co(SCN_4)]$ (R_6) are also considered selective reagents for the three derivatives, as shown in the table (4).

Table (4): Specificity of solid form color tests.

Reagents	Diazepam	Midazolam	Clobazam
$H_2[CoCl_4]$ (R_1)	+	+	-
$H_2[Co(Ac)_4]$ (R_2)	+	+	-
$H_2[Cu(Cl)_4]$ (R_3)	+	+	-
$H_2[Ni(Cl)_4]$ (R_4)	+	-	-
$H_2[Co(I)_4]$ (R_5)	+	+	+
$H_2[Co(SCN)_4]$ (R_6)	+	+	+

4- Conclusion:

We have developed a new qualitative reagents through which we can quickly and immediately detect some derivatives of the benzodiazepine group in both solid and liquid forms, through the formation of distinctive color chemical spots through a chemical reaction between the suspicious substance and special chemicals, which leads to the identification of the narcotic substance quickly and accurately. It helps direct investigations and provide the necessary evidence in drug cases. The reagents demonstrated high efficacy and selectivity compared to other pharmaceutical substances.

5- Acknowledgement

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