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RESIDUAL SOLVENTS ANALYSIS IN METRONIDAZOLE RAW MATERIAL USING HEAD SPACE GAS CHROMATOGRAPHY

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In pharmaceutical raw material (PRM), the residual solvents (RS) are residual impurities which must be controlled due to their toxicity. In this study, we report the quality control results of residual solvents impurties analysis using head space gas chromatography with flame ionization detector (HS-GC-FID) of six raw materials samples of Metronidazole marketed in Algeria. The GC is equipped with a flame-ionization detector and silica column coated with 1.8 µm layer of phase G43. The carrier gas is nitrogen with a linear velocity of 35 cm/s and a split ratio of 1:5. The column temperature is 40 °C then it rise to 240 °C. The injection temperature is 140 °C and that of detector is 250 °C. 29 organic solvents belongs to classes 1 and 2 were researched whose control is mandatory because of their carcinogenic and intrinsic toxicity, only five solvents were identified in the different samples and the methanol was quantified in M2 sample. All samples collected satisfy the test except M2 sample which contains a slight excess of methanol estimated of 14 ppm. This slight excess show that M2 sample wasn't well purified and this may be due to the difficulty of solvents complete removal.

Keywords: *Residual Solvents, HS-GC-FID, Metronidazole, Active Pharmaceutical Ingredient, Solvents-impurties.*

INTRODUCTION

In the different steps synthesis of pharmaceutical raw materials (PRM), the organic solvents are habitually used.[1] Residual solvents cannot be completely removed due to certain chemical and physical criteria.[2&3] Residual solvents (RS), that is, volatile organic impurities (VOI) are small amounts of solvents remaining in the PRM after purification processes.[3&4] Drug manufacturers should minimize residual amounts of solvents due to their toxicity to patients. Therefore, safety standards must be respected which are published in the pharmacopoeias and guidelines of the ICH.[4&5] Residual organic solvents must be controlled, therefore, if their amounts are below the limits, the PRM is compliant and if they are greater, purification is necessary.[5]

ICH has also included daily exposure limit of many solvents it has classifed these solvents into four classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard[6]. Class I solvents (which covers 5 residual solvents) are known or suspected human carcinogens and environmental hazards, the use of these solvents should be avoided. Class I solvents should be identifed and quantifed. Class II solvents (which covers 29 residual solvents) are non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Use of these solvents should be limited. Class II solvents have individual limits. Class III solvents (which covers 26 residual solvents) having low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents

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have PDEs of 50 mg or more per day. Finally, Class 4 solvents are those for which no adequate toxicological data have been found[7]**.** Therefore determination of residual solvents becomes a necessary procedure for quality control of drug substances and drug product to meet regulatory guideline and ensure patient safety[8*9]**.**

Head space gas chromatography (HSGC) is generally used to determine residual solvents because of its high separation efficiency and sensitivity for organic volatile solvents**.** However head space bounds the analysis to those solvents being evaporated from HS only, it also requires larger sample load and analysis time should be longer due to sample equilibration. Headspace sampling is preferred because of its ability to avoid direct liquid or solid injection[10]**.**

Metronidazole is a synthetic antiparasitic that is part of the list of essential drugs established by World Health Organization, manufactured by several generic laboratories in Algeria, their high rate of prescription by clinicians thanks to their numerous indications in the different infections (gynecological, urinary, digestive and respiratory,…. etc.) **(Figure 1).**[11]

In this paper, we will analysis and evaluate the residual solvents in six samples of Metronidazole raw material marketed in Algeria using HS-GC-FID.

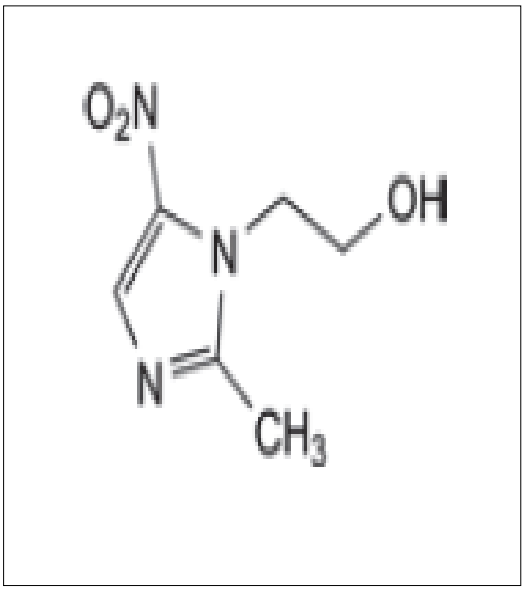

Fig. 1: Chemical structure of Metronidazole.[11]

MATERIALS AND METHODS

Six samples of an antiparasitic raw material called Metronidazole were collected from pharmaceutical producers installed in Algeria. They are labeled as follows: M1, M2, M3, M4, M5 and M6 **(Table 1).**[12]

Identification and quantification of residual solvents by HS-GC-FID[13&14]

Standards, reagents and apparatus

USP Class 1, USP Class 2 _Mix A and USP Class 2 _Mix B residual solvents standards used for peak identification were purchased from Restek (Bellefonte, USA) and Dimethyl sulfoxide produced by Riedel-de Haën Germany.

Table 1: Sampling of Metronidazole raw material.

Sample	Local Producer	Batch number	Expiration Date	Manufacturer-Supplier
M1	Lab M1	20130558	06/2017	Quimdis (France)
M2	Lab M2	09330056R	09/2019	Quimdis (France)
M3	Lab M3	20110387	02/2018	Aarti Drugs Limited (India)
M4	Lab M4	A00181	05/2017	Unknown
M5	Lab M5	119M231	10/2018	Unknown
M6	Labo M6	345P/13	11/2017	Unknown

Composition of residual solvents standard solutions

- **Class 1_USP (10-50 mg/mL):** 1,1-dichloroethene, 1, 1,1-trichloroethane, Carbon tetrachloride, Benzene and 1,2-Dichloroethane.
- **Classe 2_USP_Mix A (0,35-19,4 mg/mL):** Cyclohexane, Methylcyclohexane, trans-1,2-dichloroethene, Tetrahydrofuran, Methanol, Dichloromethane, cis-1,2-dichloroethene, Acetonitrile, Toluene, 1,4-Dioxane, Ethylbenzene, p-Xylene, m-Xylene, Isopropylbenzene, o-Xylene and Chlorobenzene.
- **Classe 2_USP_Mix B (50-290 µg/mL):** n-Hexane, Nitromethane, Chloroform, 1,2-Dimethoxyethane, Trichlorethylene, Pyridine, 2-hexanone and tetralin.

A Gas Chromatograph (GC-2010 Plus-Shimadzu Japan) coupled to flame ionization detector (FID) and headspace extraction sampler "HS" (Auto sampler AOC-5000 Plus-Shimadzu Japan).

Analysis protocol[13,14&15]

- **Class 1 Standard Stock Solution:** Prepared from USP_Class 1 Residual Solvents at concentration of 10^{-5} mL/mL.
- **Class 1 Standard Solution**: Prepared from Class 1 Standard Stock Solution at concentration of 0,2 mL/mL in headspace vial.
- **Class 2 Standard Stock Solution A:** Prepared from USP Residual Solvents Class 2_Mixture A at concentration of 10^{-2} mL/mL in headspace vial.
- **Class 2 Standard Stock Solution B:** Prepared from USP Residual Solvents Class 2_Mixture B at concentration of 10^{-2} mL/mL in headspace vial.
- **Class 2 Mixture A Standard Solution**: Prepared from Class 2 Standard Stock Solution A at concentration of 0,5 mL/mL in headspace vial.
- **Class 2 Mixture B Standard Solution**: Prepared from Class 2 Standard Stock Solution B at concentration of 5 mL/mL in headspace vial.
- **Test Stock Solution**: Prepared from each Metronidazole sample at concentration of 10 mg/mL in headspace vial.
- **Test Solution**: Prepared from Test Stock Solution at concentration of 5 mL/mL in headspace vial.
- **Class 1 System Suitability Solution**: Prepared from Class 1 Standard Stock Solution at concentration of 0,2 mL/mL in headspace vial.

Procedure A of identification

The Headspace Operating Parameters are illustrated in Table 2.

Table 2: Headspace operating parameters[13&14]

Operating parameters	Operating conditions
Equilibration temperature (°C)	80
Equilibration time (min)	60
Transfer-line temperature (°C)	85
Syringe temperature (°C)	80-90
Pressurization time (S)	≥ 60
Injection volume (mL)	1
Carrier gazs	Nitrogen or helium at an appropriate pressure

Procedure C of Methanol quantification in M2 sample[13&14]

The chromatographic and headspace conditions were set in the same way as the identification procedure A.

Methanol Standard Stock Solution (150 ppm)

Prepared from USP_Methanol Standard at concentration of 150 ppm.

Methanol Standard Solution

Prepared from Methanol Standard Stock Solution at concentration of 0,2 mL/mL.

Spiked Test Solution M2

Add 1 mL of Methanol Standard Stock Solution to 5 mL of Test Stock Solution M2 in headspace vial.

Calculate the Methanol Residual Solvent amount in M2 by the formula

$$\text{Residual Solvant Content (ppm)} = 5 \times \frac{\text{C (µg/mL)}}{\text{W (g)}} \times \frac{\text{A1}}{(\text{A2} - \text{A1})}$$

C: concentration of Methanol Standard Stock Solution (µg/mL)
W: sample weight (g)
A1: Methanol peak Area in the Test Solution
A2: Methanol peak Area in the Spiked Test solution**.**

RESULTS AND DISCUSSION

Procedure A of identification

System compliance

Solvents Identification: the chromatograms obtained with the Standard Solutions (Class 1, Class 2_Mix A and Class 2_Mix B) **(Figure 2, 4, 6)** and the typical chromatograms supplied with Standard Solutions **(Figure 3, 5, 7)** are comparable, which allowed us to identify the respective peaks corresponding to solvents of each class with their retention times **(Table 3)**.

Table 3: Retention time of solvents.

Solvent	Retention time (min)
Class 1 solvents	
1,1-Dichloroethene	06.283
1, 1,1-trichloroethane	14.572
Tetrachloromethane	15.775
Benzene	17.007
1,2-Dichloroethane	17.007
Class 2_Mix A solvents	
Methanol	4.285
Acetonitrile	5.800
Dichloromethane	7.556
trans-1,2-dichloroethene	8.412
cis-1,2-Dichloroethene	12.128
Tetrahydrofuran	13.565
Cyclohexane	15.12
Methylcyclohexane	22.649
1,4-Dioxane	23.730
Toluene	26.677
Chlorobenzene	30.394
Ethylbenzene	30.661
m-Xylene	30.927
p-Xylene	30.927
o-Xylene	31.811
Isopropylbenzene (Cumene)	32.604
Class 2_Mix B solvents	
n-Hexane	9.444
Nitromethane	12.562
Chloroform	13.657
1,2-imethoxyethane	17.522
Trichloroethene	21.631
Pyridine	26.668
2-Hexanone	28.584
Tetralin	38,596

Co-elution of Benzene and 1,2-dichloroethane which were eluted at the same retention time (TR: 17.007 min) (Figure 2).

Co-elution of m-Xylene and p-Xylene which were eluted at the same retention time (TR: 30.927 min) (Figure 4).

Signal-to-noise ratio: the signal-to-noise ratio of 1,1,1-trichloroethane peak is 8.82, which is greater than the limit required by the USP (at least 5). The signal-to-noise ratio of the following peaks: (1,1-dichloroethene, 1,1,1-trichloroethane, tetrachloromethane and benzene / 1,2-dichloroethane) of System Suitability Solution are respectively: 8.98, 8.82, 3.07 and 10.09. These values are according to the standard required by the USP (minimum 3).

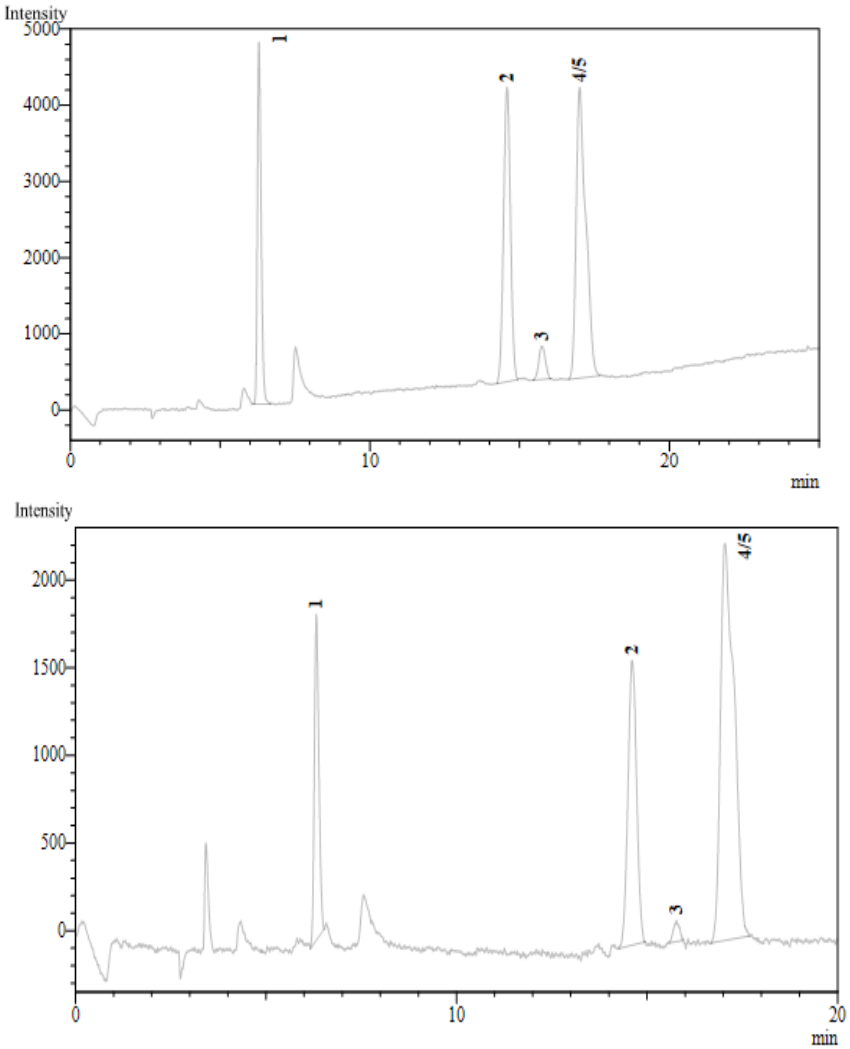

Fig. 2: Chromatograms of class 1 standard solution and class 1 system suitability solution.

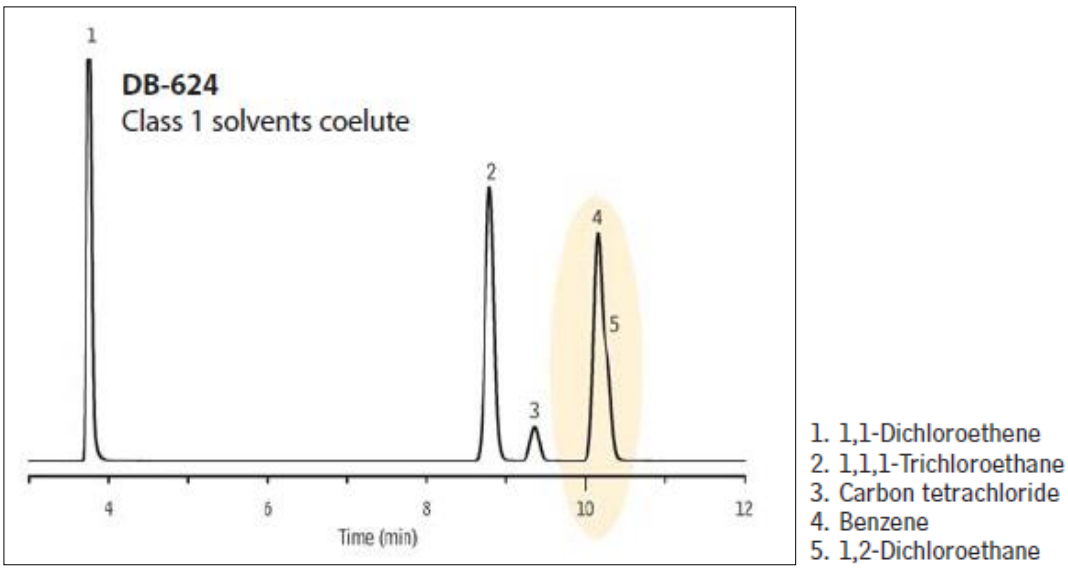

Fig. 3: Typical chromatogram of class 1_USP standard solution.[16]

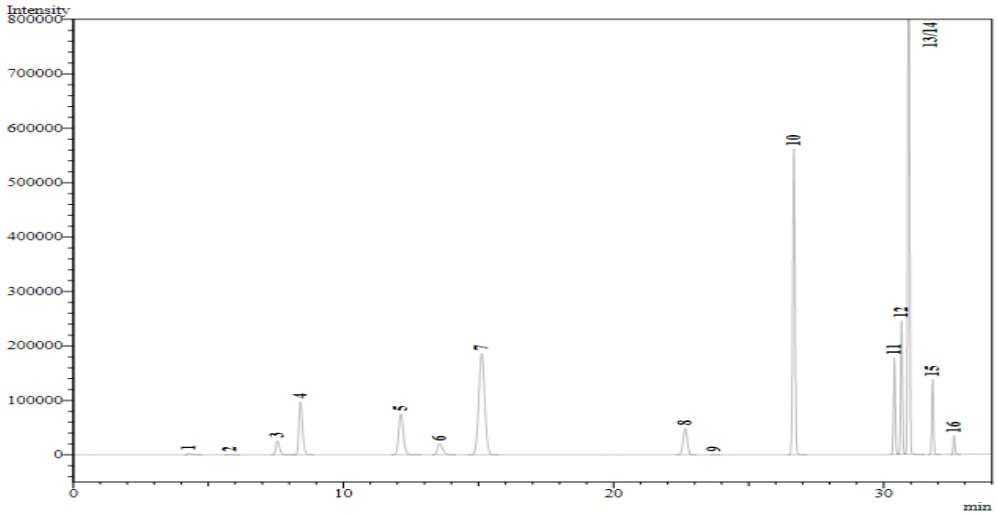

Fig. 4: Chromatogram of class 2_mix a standard solution.

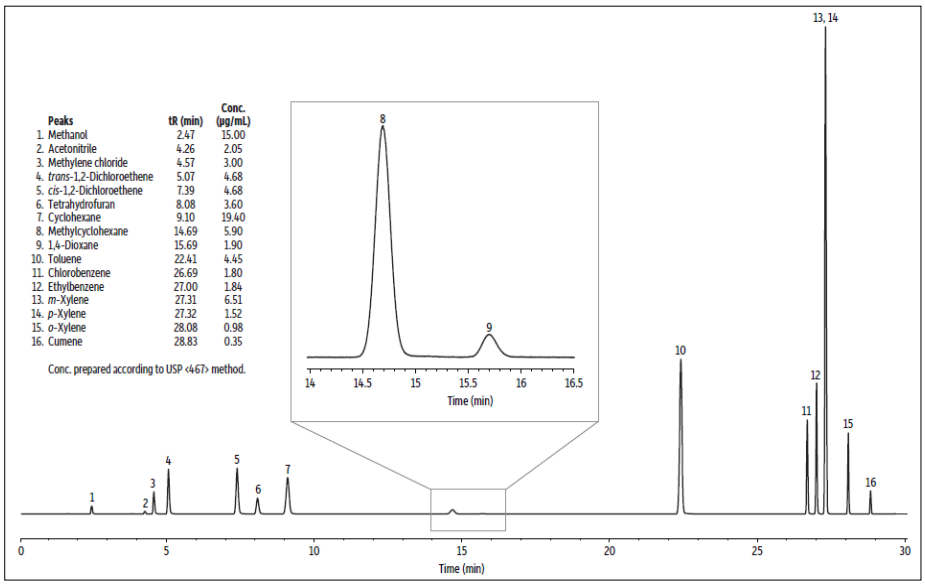

Fig. 5: Typical chromatogram of class 2_Mix A standard solution.[17]

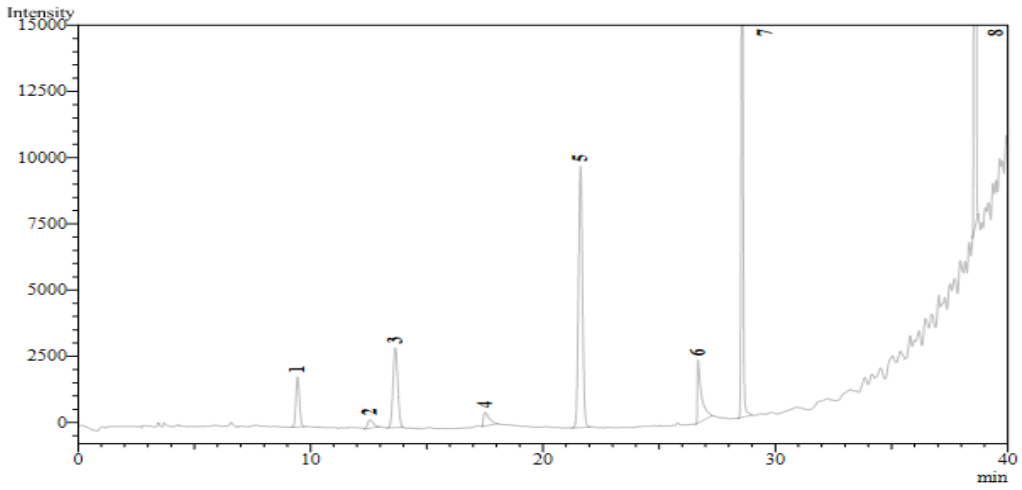

Fig. 6: Class 2_Mix B standard solution chromatogram.

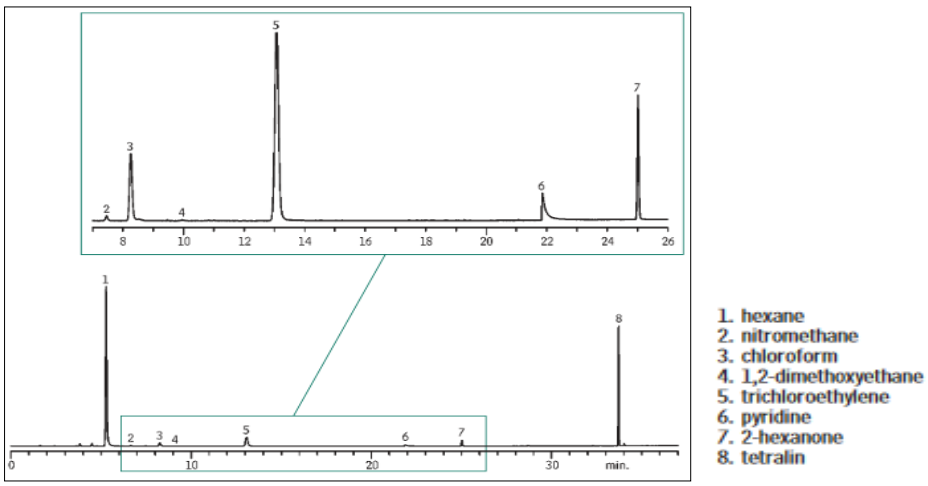

Fig. 7: Typical chromatogram of class 2_Mix B standard solution.[18]

Resolution: the resolution between acetonitrile peak and methylene chloride peak is 6, value conform to the standard (at least 1.0).
In conclusion, the system is compliant.

Samples Analysis

M3 sample: five peaks are detected, methanol, acetonitrile, 1,1-dichloroethene, dichloromethane and tetrahydrofuran **(Figure 9)** respectively, they have the following surfaces (2852 µV.min, 4765 µV.min, 458 µV.min, 22345 µV.min and 10070 µV.min) which are lower than those of the corresponding standards (33227 µV.min, 6194 µV.min, 970 µV.min, 252054 µV.min and 277962 µV.min). So M3 sample satisfies the test.

M4 sample: dichloromethane peak was appeared **(Figure 9),** having area of 4116 µV.min, lower than that of the corresponding standard (252054 µV.min). So M4 Sample satisfies the test.

M6 sample: methanol peak was detected **(Figure 10)**, having surface of 2612 µV.min, lower than that of the corresponding standard (33227µV.min). So M6 Sample satisfies the test.

M2 sample: only one peak was detected, that of Methanol **(Figure 8)**, having surface of 33409 µV.min, higher than that of the corresponding standard (33227 µV.min). So M2 sample doesn't satisfy the test. A confirmation and quantification of methanol is mandatory.

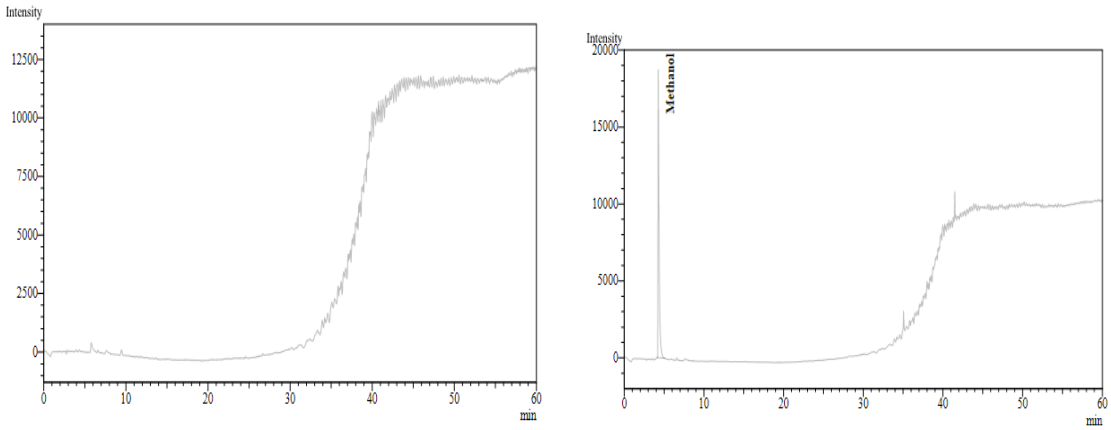

Fig. 8: Chromatograms of M1 and M2 samples.

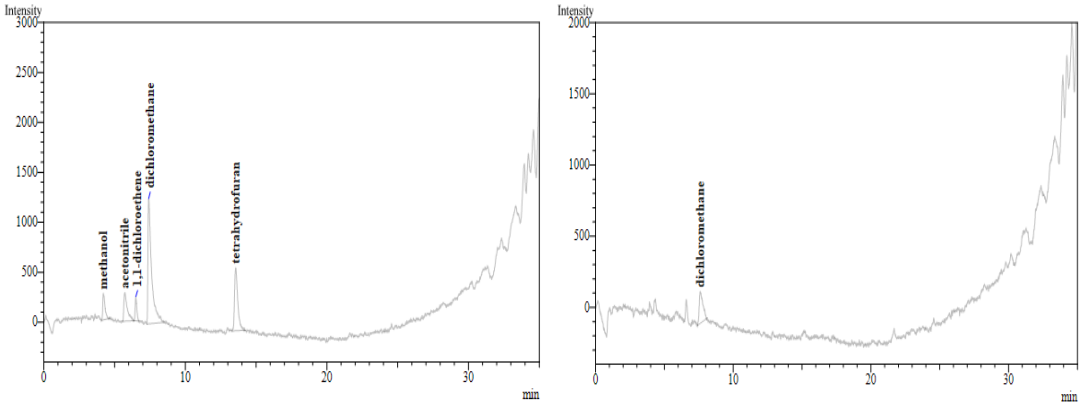

Fig. 9: Chromatograms of M3 and M4 samples.

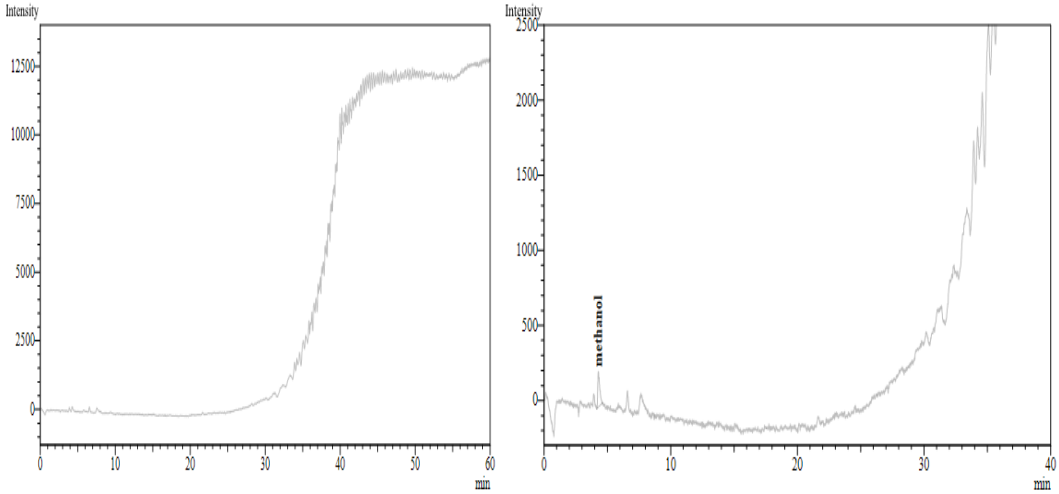

Fig. 10: Chromatograms of M5 and M6 samples.

Procedure C of Methanol quantification in M2 sample.

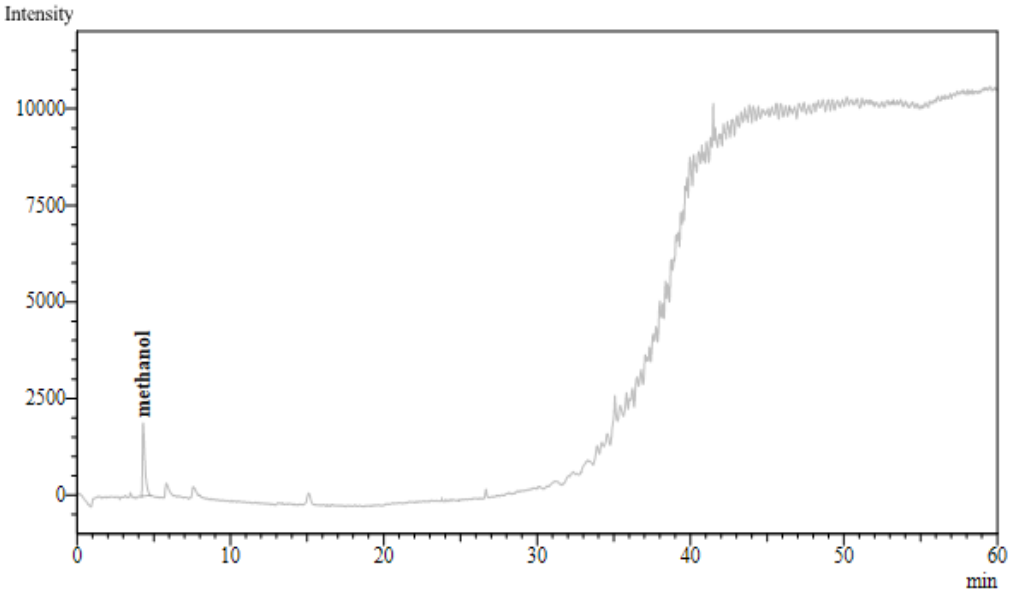

Fig. 11: Methanol standard solution chromatogram.

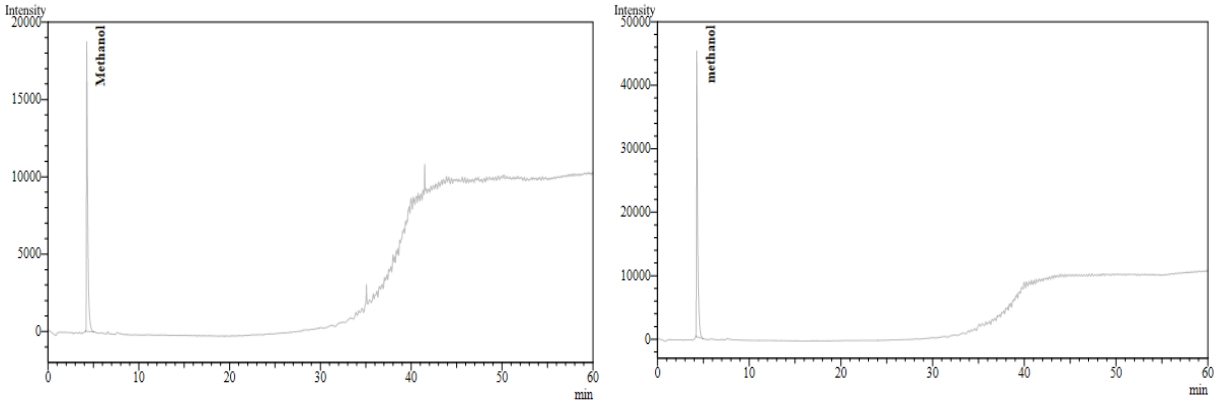

Fig. 12: M2 sample and M2 spiked sample chromatograms.

Table 3: Methanol content result in M2 sample.

Sample	Sample Weight (g)	Methanol Concentration (µg/mL)	Methanol Area in test solution A1 (µV.min)	Methanol Area in spiked test solution A2 (µV.min)	Methanol Content (ppm)	Allowed Limit (ppm)
M2	0,2501	150	33405	66640	**3014,14**	3000

The methanol content in M2 sample is estimated at 3014 ppm, this value is greater than the allowed limit of 3000 ppm **(Table 3)**. Knowing that Methanol is used as solvent in synthesis or purification process, this excess in methanol shows that the sample hasn't been well purified, however it should be remembered that organic solvents aren't always easy to eliminate.

Conclusion

29 organic solvents belongs to classes 1 and 2 were researched in six samples of Metronidazole Active Pharmaceutical Ingredients (API) whose control is mandatory because of their carcinogenic and intrinsic toxicity, only five solvents were identified in the different samples and the methanol was quantified in M2 sample. All samples collected satisfy the test except M2 sample which contains a slight excess of methanol estimated of 14 ppm. This slight excess show that M2 sample wasn't well purified and this may be due to the difficulty of solvents complete removal. The HS-GC-FID technique used showed that the identified solvents differ from one sample to another of the same molecule. This shows that manufacturers don't often use the same solvents to produce the same API, which justifies that residual organic solvent tests aren't usually mentioned in the specific monographs.

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Conflict Of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for content and writing of the paper.

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نشرة العلوم الصيدليـــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تحليل المذيبات المتبقية في مادة ميترونيدازول الخام باستخدام كروماتوجرافيا غاز مساحة الرأس

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في المواد الخام الصيدلانية (PRM، المذيبات المتبقية) (RS) هي شوائب متبقية يجب التحكم فيها بسبب سميتها. في هذه الدراسة ، قمنا بالإبلاغ عن نتائج مراقبة الجودة لتحليل شوائب المذيبات المتبقية باستخدام كروماتوجرافيا غازات الرأس مع كاشف تأين اللهب (HS-GC-FID) لست عينات من المواد الخام من Metronidazole يتم تسويقها في الجزائر. تم تجهيز GC بكاشف التأين باللهب وعمود السيليكا المطلي بطبقة ١,٨ ميكرومتر من المرحلة .G43 الغاز الحامل عبارة عن نيتروجين بسرعة خطية ٣٥ سم/ثانية ونسبة انقسام ١: ٥. درجة حرارة العمود ٤٠ درجة مئوية ثم ترتفع إلى ٢٤٠ درجة مئوية. درجة حرارة الحقن ١٤٠ درجة مئوية ودرجة حرارة الكاشف ٢٥٠ درجة مئوية. تم بحث ٢٩ مذيبًا عضويًا ينتمي إلى الفئتين ١ و ٢ ، والتي تعد السيطرة عليها أمرًا إلزاميًا بسبب سُميتها الذاتية المسببة للسرطان ، وتم تحديد خمسة مذيبات فقط في العينات المختلفة وتم تحديد كمية الميثانول في عينة M2 جميع العينات التي تم جمعها تحقق الاختبار باستثناء عينة M2 التي تحتوي على فائض طفيف من الميثانول يقدر بـ ١٤ جزء في المليون. تظهر هذه الزيادة الطفيفة أن عينة M2 لم يتم تنقيتها جيدًا وقد يكون هذا بسبب صعوبة الإزالة الكاملة للمذيبات.