DETECTION OF COUNTERFEIT MEDICINES: FROM HIGH-FIELD TO LOW-FIELD NMR

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Introduction

The proliferation of counterfeit and poor-quality drugs is a major public health problem, especially in developing countries lacking adequate resources to effectively monitor their prevalence. Falsified medicines constitute a major challenge for analytical laboratories to detect and characterize them.

In this study, counterfeit antimalarial, impotence and abortive medicines were analysed using high-field and low-field NMR demonstrating the ability of the technique for detecting poor quality drugs and unexpected active pharmaceutical ingredients (API).

Experimental part

Sample preparation

For each formulation, the tablets were powdered and the powder was analysed. The powder was dissolved in 1 mL of CD_3CN:D_2O (80:20 v/v) or MEOH-d4 and transferred to a 5 mm NMR tube. A solution of sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (TSP) was added as an internal chemical shift and quantification reference.

NMR analysis

• 500 MHz

^{1}H NMR experiments were run on a Bruker Avance 500 spectrometer operating at 500.13 MHz, equipped with a 5 mm cryoprobe at 298K. For qualitative purpose (profiling), NMR acquisition parameters were as follow: 90° RF pulse, TD: 32K, D1: 1 s, number of scans (NS): 16 (1 min). For quantitative analysis, the same conditions were used except the RF pulse (30°), the relaxation delay lengthened to 6 s, and the NS: 64 (11 min).

• 60 MHz

^{1}H NMR experiments were run on the bench-top cryogen-free 60 MHz NMR spectrometer Pulsar™ (Oxford Instruments) in deuterated methanol. For profiling, NMR acquisition parameters were as follows: 90° RF pulse, TD: 16K, D1: 2 s, NS: 256 (22.5 min). For quantitative analysis, the same conditions were used except the RF pulse (30°) and the NS: 512 (45 min).

MS analysis

The powder was dissolved in CH_3CN:H_2O (80:20 v/v) and analysed directly using a Waters XEVO G2 QTOF mass spectrometer under positive and negative ionization modes.

Drugs for erectile dysfunction

Counterfeit drugs commercialized as copies of Viagra (sildenafil), Cialis (tadalafil) and Levitra (vardenafil) were analysed. Among the 40 formulations analysed, 26 were correct, 8 contained unexpected API and for 6 the dosage was incorrect.

Two cases of fake formulations commercialized as vardenafil are presented below. “Vardenafil n°1” contains sildenafil (Figure 2) and “Vardenafil n°2” contains tadalafil and diclofenac, a nonsteroidal anti-inflammatory drug (Figure 3).

« VARDENAFIL » N° 1

Counterfeit

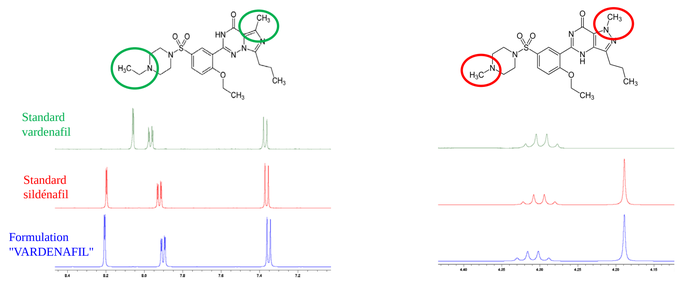

Figure 2. Part of the ^{1}H NMR spectrum of a formulation sold as Vardenafil compared to ^{1}H NMR spectra of standard vardenafil and sildenafil (500 MHz, CD_3CN:D_2O (80:20))

« VARDENAFIL » N° 2

Counterfeit

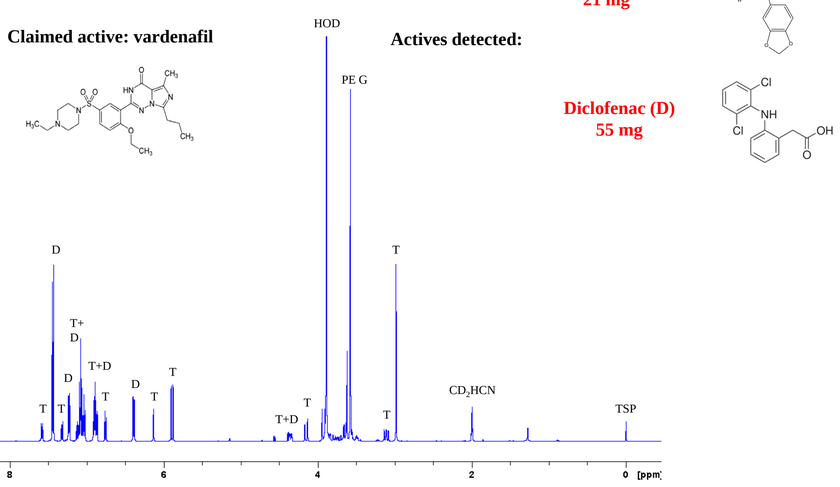

Figure 3. ^{1}H NMR spectrum of a formulation sold as Vardenafil (500 MHz, CD_3CN:D_2O (80:20)). T: Tadalafil, D: Diclofenac

Quantitative analysis with high-field and low-field ^{1}H NMR

The absolute quantification by the internal standard method was carried out for 3 formulations using both high-field (500 MHz) and low-field (60 MHz) NMR.

	API announced	API found	500 MHz	60 MHz	
			mg/tablet	mg/tablet	% error
Levitra	vardenafil	sildenafil	21.0	22.6	7.6
		tadalafil	4.5	5.2	15.6
Vardenafil	vardenafil	sildenafil	44.9	48.3	7.6
Cialis	tadalafil	sildenafil	27.1	28.6	5.5

Antimalarial drugs

Antimalarial drugs are among the most counterfeit drugs. We analysed antimalarial drugs claimed to contain artesunate, or the combination therapies dihydroartemisinine/piperaquine or artemether/lumefrantrine. Among the 20 formulations analysed, 8 were correct, 7 contained no API, and 5 contained a wrong API.

The High- and Low-Field ^{1}H NMR spectra of 3 formulations of “artesunate” collected in South-East Asia are shown in Figure 1. One medicine contains artesunate and the two others the analgesics paracetamol or dypirone.

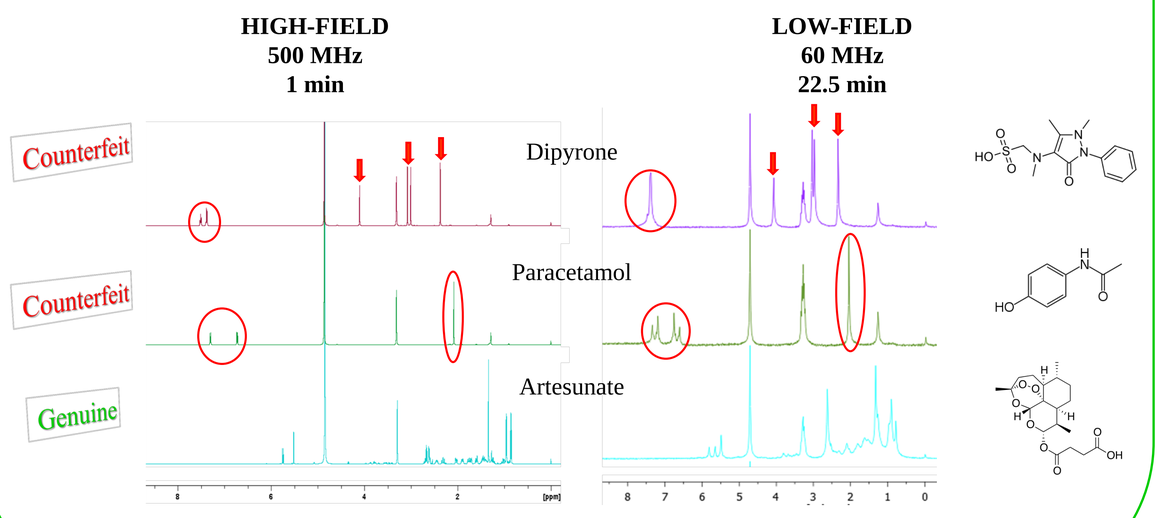

Figure 1. ^{1}H NMR spectra of genuine and counterfeit formulations of the antimalarial drug artesunate (solvent: MeOD-d4).

A morning-after pill case: Pregniloc

The case of the morning-after pill “Pregniloc” bought on a website is reported below. This pill is sold as a blister with 2 pills, each supposed to contain 0.75 mg of levonorgestrel as API. The ^{1}H NMR spectrum of this medicine is presented in Figure 4A. The excipients lactose, PEG (macrogol) and benzoate along with the API levonorgestrel were detected. Two other API were identified, paracetamol and tramadol. The very low level of tramadol precluded its quantification by ^{1}H NMR.

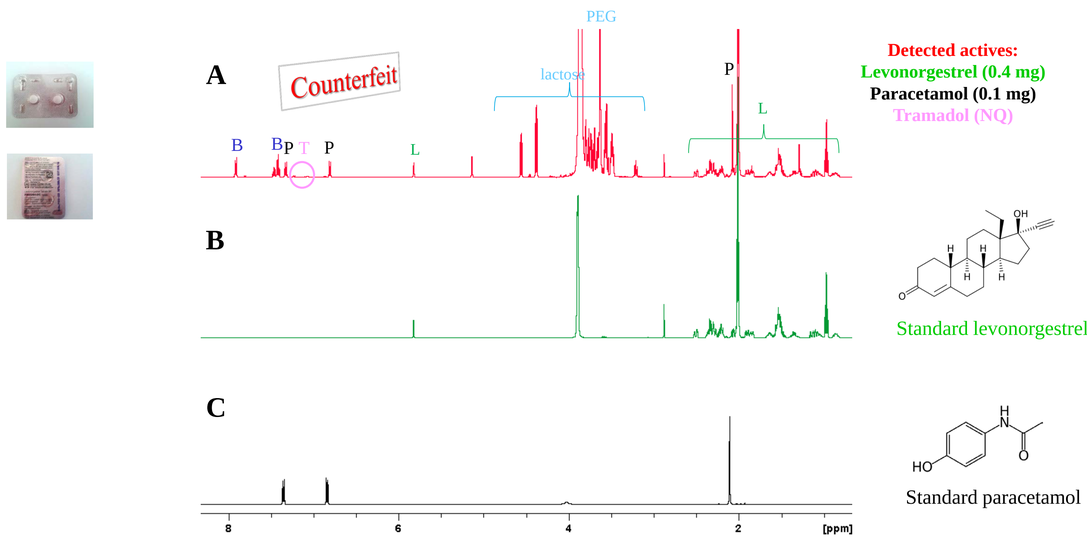

Figure 4. ^{1}H NMR spectra of (A) a Pregniloc pill, (B) standard levonorgestrel, (C) standard paracetamol (solvent CD_3CN:D_2O (80:20)). L: levonorgestrel, P: Paracetamol, B: Benzoate, T: Tramadol.

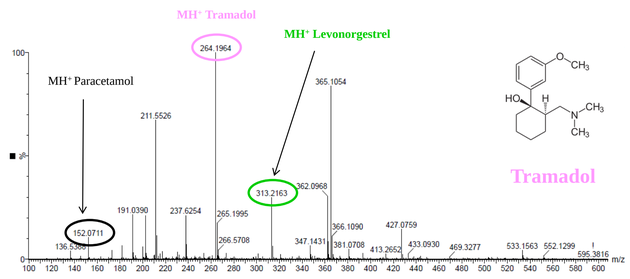

Figure 5. MS spectrum in positive ionization mode (ESI) of the Pregniloc formulation.

The presence of the 3 actives was confirmed by mass spectrometry (MS) (Figure 5). An error <1.5 ppm is measured between the experimental and calculated values.

Conclusion

In this study we demonstrate that ^{1}H NMR allows to fully characterize counterfeit drugs. Different kinds of counterfeiting were observed: drugs without API, underdosed and overdosed medicines, and drugs with wrong API(s). If the main drawback of high-field NMR is the cost of the equipment, we demonstrate the ability of a low-field bench-top cryogen-free NMR spectrometer for detection and quantification of counterfeit drugs. The introduction of such spectrometers in quality control laboratories has to be considered especially in the field of counterfeit drug detection.

Acknowledgements

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