International Journal of Medicine Research
www.medicinesjournal.com
ISSN: 2455-7404
Received: 15-10-2021, Accepted: 30-10-2021, Published: 16-11-2021
Volume 6, Issue 4, 2021, Page No. 21-22

Standardization of some plant-based medicinal products registered in Dr Congo by high-performance liquid chromatography (HPLC): A preliminary study

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Abstract
This study was carried out as part of the standardization of herbal medicines registered in DR Congo. In order to ensure the quality control of registered herbal medicines, We have used a separative technique which is HPLC-UV capable of tracing several active molecules found from these herbal drugs. The column used is X-Brigde, C18, 250 X 4.6mm, 5um. The column temperature was set at 25 ° C and we used 4 wavelengths 220nm, 254nm, 280nm and 280nm. The results obtained show that the chromatographic peaks are well separated and the biomarkers have been fixed for the identification and the quantitation of these products.

Keywords: herbal medicines, standardization, HPLC

Introduction

The quantity of active substance in a pharmaceutical product has always been a key element in the quality of the medicine. This importance is such that one cannot conceive the analysis of a medicinal product without knowing the content of active principles. Therefore, if the active principle of a herbal medicine is not known, the content of its biological indicators is an important factor in the quality of the product and the reproduction of its activity.

The quantity of active substances or at least the quantity of biological indicators of the herbal medicine is an important characteristic of its quality.

The chromatographic and spectroscopic methods used for its study are also used for the quantification of active ingredients or biological indicators. Chromatography is the most widely used method for quality control of herbal medicines.

Among these techniques, high performance liquid chromatography (HPLC) is regularly used as quantitative analysis methods because of its high precision, its possibility of using several stationary phases and its possibility of being associated with many detectors (UV and NMR)(Dotsé et al., 1997; Nicaise, 2013; Kamboj, 2012; Liang et al., 2009; Yongyu et al., 2011; Hamilton et al, 1982). It is in this capacity that we referred to HPLC to develop a method for quantifying biological indicators in herbal medicines. (Sethi P. D and Sethi R., 2012).

Materiel and Methods

Different batches of five herbal medicines registered with the DPM (Department of Pharmacy and Medicines) of the Ministry of Public Health were collected from different manufacturers and purchased in a few pharmacies open to the public for our research in different pharmaceutical forms. These are Manadiar (tablets and suspension) and Manalaria (tablets and suspension).

Analyzes were performed using an Merck Hitach series HPLC chain, and an diode array UV detector (Antwerp, Belgium). The chromatographic column was used, an XBridge C18 (250 × 4.6 mm i.d.; 5 µm particle size) from Waters (Massachusetts-MA, USA).

The flow rate of the mobile phase was set at 1.0ml / min and the detection was carried out at 220nm, 254nm, 280nm and 320nm.

The following conditions were used for all compounds

Mobile phase: Methanol (MeOH) and trifluoroacetic acid (TFA)

Table 1: Elution gradient

Time (min)	Mobile phase	
	MeOH (%)	TFA 0.05%
0.0	20	80
45	70	30
55	70	30
56	20	80
65	20	80

These experimental conditions were also used for the recording of all quantitative analytical chromatograms of samples sprayed and dissolved in methanol (tablets), suspensions diluted in methanol and standards.

Results and discusion

Results

For Manadiar tablets and suspension, the substances analyzed were mangiferin and quercetin. For Manalaria tablets and suspension, the substances analyzed were vitexin and chrysophanol. All of these compounds were detectable by HPLC under the experimental

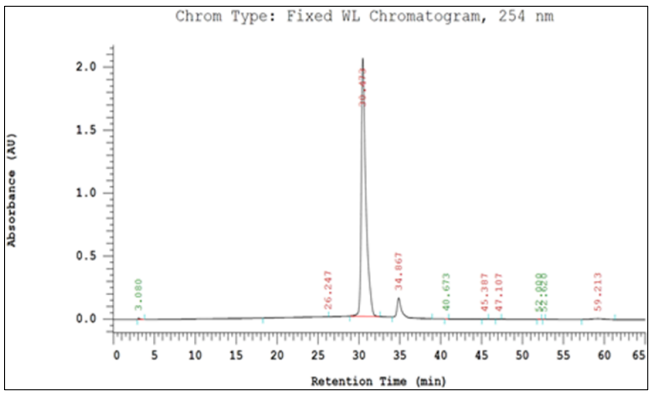

Fig 1: HPLC chromatograms of mangiferin at 254nm

Comparison of the peak areas of the references and the samples directly gives the concentrations of the biological markers in Manadiar and Manalaria

Table 2: Surface areas and retention time of mangiferin et quercetin contained in *Manadiar tablets*

ʎ (nm)	Mangiferin (tr 30.493 min)	Quercetin (tr 58.940 min)
	Area	Area
220	15114316	8760528
254	24649825	9760720
280	6554499,0	3525565
320	14084958	3992248

Table 3: Surface areas and retention time of mangiferin and quercetin contained in *Manadiar tablets*

ʎ (nm)	Mangiferin (tr 30.567min)	Quercetin (tr 59.033 min)
	Aires	Aires
220	6053827	845709
254	9959210	781299
280	2810025	308214
320	5594969	276326

Table 4: Content of mangiferin et quercetin in 250 mg of *Manadiar tablets*

ʎ (nm)	mangiferin (mg)	quercetin (mg)
220	1.5842	0.8743
254	1.5989	0.8655
280	1.5400	0.8805
320	1.5925	0.8485

Table 5: Content of vitexin and chrysophanol in 250 mg of *Manalaria tablets*

ʎ (nm)	Vitexin (mg)	Chrysophanol (mg)
220	2.1110	1.2988
254	1.7440	0.9205
280	1.2765	0.7802
320	0.6660	0.7838

Discusion

The concentrations of the various biological markers were determined (quercetin and mangiferine in Manadiar tablets and suspension; chysophanol and vitexin in Manalaria tablets and suspension) by electronic comparison of the peak areas of the reference substances and of the samples by HPLC. These values are used to identify these drugs based on plnate.

Conclusion

The main objective was to develop an HPLC method for the analysis of herbal drugs marketed in the DRC.

In this study we quantified the biological markers present in herbal medicines with references certified by HPLC. These results thus provide both the manufacturer and the regulatory authority with instruments of primary importance for the safety of the use of these drugs. In this method is an advance for the quality control of these drugs

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