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Development, transfer and validation of a HPLC-DAD method for analysis of six herbal medicines marketed in D.R. Congo: Fingerprinting and stability study

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ABSTRACT

Context: Phytomedicines, also known as improved traditional medicines (ITMs), are increasingly used in the management of diseases throughout the world. While quality control widely known to be an essential tool that can ensure the efficacy and safety of health products, most ITMs lack appropriate analytical methods for their control. The aim of this study was to develop a reliable analytical method for the quality control of ITMs commonly used in the Democratic Republic of the Congo (D.R. Congo). For this purpose the method needs to be validated and transferred.

Objectives: The objective of this research was to develop, transfer, and validate an affordable, rapid and easily implementable analytical method by HPLC-DAD for the quality control of herbal medicines marketed in the Democratic Republic of the Congo. Also, to determine these drugs fingerprint and their stability under accelerated conditions.

Methods: Chromatographic separation was achieved using two chromatographic columns, an XBridge C18 (250 × 4.6 mm i.d.; 5 µm particle size) and an XBridge C18 (100 × 4.6 mm i.d.; 3.5 µm particle size), column maintained at 30 °C. The mobile phase consisted of a gradient mixture of mobile phases A (acetonitrile) and B (aqueous solution of trifluoroacetic acid 0.05%) pumped at 1.0 mL/min. UV detection was performed at 280 nm. The method using the short column was validated using the total extracts as references. The strategy of total error was used to decide on the reliability of the method taking into account the acceptance limits fixed at ± 10%.

Results: The validation results show that the developed HPLC-DAD method presented an adequate trueness, linearity, precision and accuracy. Two extra peaks found in the finish products did not interfere with phytomarkers, thus confirming the selectivity of the developed method as appropriate chromatographic profiles. The method was successfully used to determine the content of the phytomarkers in six ITMs. Furthermore, we could notice that the ITMs submitted to the accelerated degradation studies remained chemically stable over six months of harsh treatments.

Conclusion: A generic method of analysis of herbal medicines was developed using HPLC-DAD, and subsequently validated before being applied in a stability study of these drugs. The proposed method holds the promise of providing a new tool for quality product development in the field of traditional herbal medicines.

1. Introduction

Over several decades the humans around the world have used elements of their environment, in particular plants, to treat a wide range of diseases. The literature informs that 66 to 85% of the world's population, mainly in developing countries use plants as an alternative to

Abbreviations: CRMTA, Centre for Research on Improved Traditional Medicines; CS, calibration standard; DIA, Diazostimul®; DRC or D.R. Congo, Democratic Republic of the Congo; HPLC-DAD, High Performance Liquid Chromatography-Diodine Array Dectector; ICH Q1A, International Conference on Harmonization Unit Q1A; ITM(s), Improved Traditional herbal medicines; MEY, Meyamycin®; NSA, N'sansiphos®; TFA, trifluoroacetic acid; VS, validation standard; ZIN, Zinginalis®.

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Fig. 1. Brands presentations (NZI: Zinginalis, MEY: Meyamycin, NSA: Nsansiphos, DIA: Diazostimule).

Table 1
List of used drugs grouped according to their batches.

Drugs	Pharmaceutical presentation (form)	Batches
DIA	Tablets	DZC1, DXC3, DZC3 and DZC5
MEY	Tablets	MLC5, MXC7, MWC7 and MYC8
	Syrup	MLS1, MXS1, MYS3 and MWS4
NZI	Tablets	ZYC1, ZZC1, ZZC2 and ZYC2
NSA	Tablets	NZC1, NZC2 and NZC3
	Syrup	NZS1, NZS2, NZS3 and NZS4

heal themselves (Mboni et al., 2020, Longanga et al., 2020).

The Democratic Republic of Congo (DRC) is one of the sixteen countries around the world qualified as mega biodiversity (high rate of endemism). This is attributed to the vastness of its territory (234.5 million hectares) and the variety of climatic conditions influencing its biodiversity. Therefore, traditional medicinal practices involving the use of plants are commonly accepted in DR Congo as the case for other countries of Central Africa (Hugéab et al., 2020, Ngoyi et al.,2015, Basimine et al., 2021).

Plants from Congolese flora are used by the local population through traditional practitioners to treat a range of diseases. These medicinal recipes are converted to Improved Traditional Medicines (ITM) by pharmacists, who apply scientific galenic knowledge to improve their presentation to guarantee the ITM efficacy and safety as well as extended shelf lives. Among the ITMs commonly used in DRC, we can cite as examples:

- Ø Meyamycin® (MEY) tablet and syrup used against acute or chronic diarrhea of infectious, amoebic, food-toxic origin, yeasts and as poison antidote;
- Ø N'sansiphos® (NSA) tablet and syrup used against malaria in all its trophozoic and febrile forms;
- Ø Diazostimul® (DIA) tablet used in case of sexual impotence and
- Ø Zinginalis® (ZIN) tablet used against hemorrhoids.

However, the therapeutic success of these drugs could be compromised by their quality control insufficiency due to lack of reliable analytical methods. This situation leads among other things, to the impossibility to undertake any study such as stability studies, dissolution test, and on the other hand to prevent the health against risk related to counterfeiting exposure. Given the well-known harmful consequences of counterfeiting on public health, it is urgent to protect the population by developing reliable analytical methods capable of ensuring the quality control of ITMs while detecting and discriminating fake medicines against genuine. Also, during the product transfer, the management system needs to take into account its complexicity to ensure that all responsibilities are defined for each involved part and important informations are also transferred. The quality system in pharmaceutical industry should ensure that the product meets required quality in terms of analytical method performance in routine, the appropriate set for analysis. Quality control is not related only to the laboratory practices, it should be considered also for decisions to be taken regarding the product quality. In this context, several analytical methods were developed (Habyalimana et al., 2017, Mbinze et al., 2013, Tshisekedi et al., 2016, ICH Q10, 2009, ASEAN Guidelines Chap.7, 2016).

Earlier in twentieth century, the liquid chromatography was discovered and until today this is used as analytical technique for separation, identification and quantification of components in mixture. As an analytical technique, HPLC use is associated to huge success due to its performance (reproducibility, selectivity, resolution, etc.) that can be

Table 2
Gradient mode by different column (based on their length).

Long column Time (min)	Acetonitrile (%)	Trifluoroacetic acid 0,05%	Short column Times (min)	Acetonitrile (%)	Trifluoroacetic acid 0,05%
0,0	5	95	0.0	5	95
5	5	95	1.7	5	95
25	95	5	10.1	95	5
40	95	5	11.5	95	5
41	5	95	11.6	5	95
60	5	95	17.1	5	95

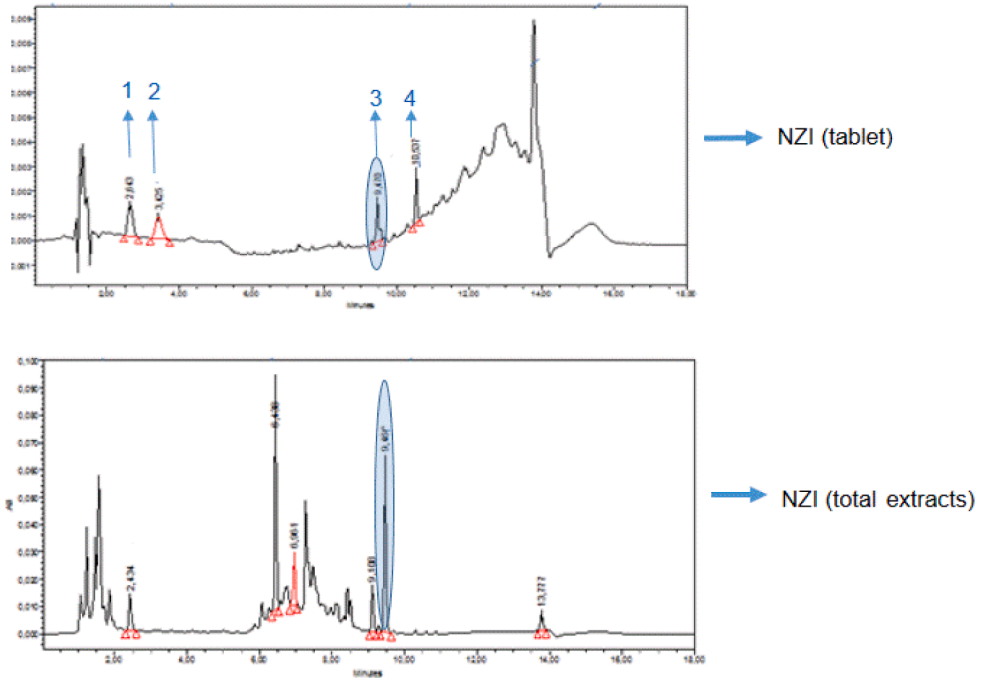

Fig. 2. Zinginalis (tablets and total extracts) Chromatograms.

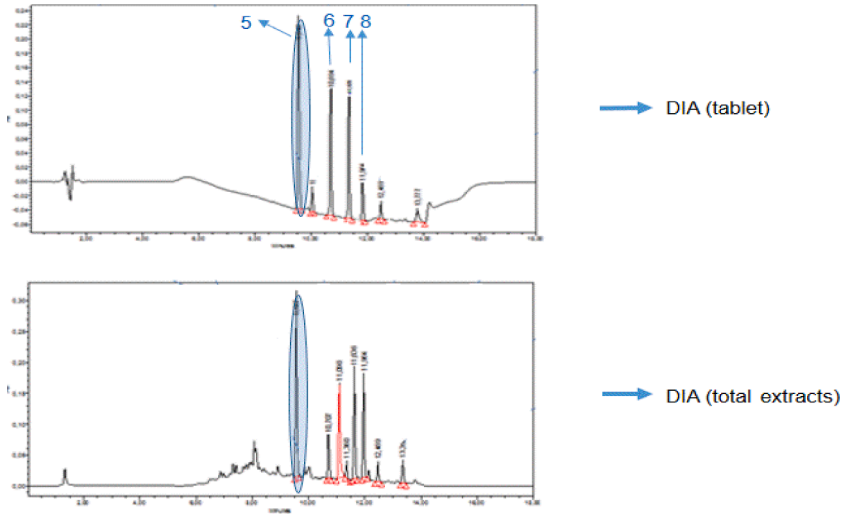

Fig. 3. Diazostimule (tablets and total extracts) chromatograms.

useful to operate in wide range of conditions for several compounds. This is commonly coupled with an UV detector unit that generates rays of specific wavelength to the eluting sample absorption (Ghanjaoui et al., 2020).

Therefore, the aim of this study was firstly to develop a generic HPLC-DAD method as a chromatographic profile for the identification of the compound of interest in the ITM and secondly to use the developed method for the quality control of ITM and fight against the counterfeiting practice. Thus, six ITM manufactured by the Centre for Research on Improved Traditional Medicines (Centre de Recherche des Médicaments Traditionnels Améliorés in French, CRMTA) of DRCongo were selected. Thirdly we project to apply the validated method as a stability indicated method for the benefit of marketing authorization.

2. Experimentation

2.1. Chemicals and reagents

HPLC grade acetonitrile and trifluoroacetic acid (TFA) 99.8% were supplied by Merck laboratory (Darmstadt, Germany). The ultra-pure quality water was produced using a Milli-Q Plus 185 brand device (Massachusetts-MA, USA). The different batches of Zinginalis® tablets and sirup, Meyamycine® tablets and sirup, Diazostimul®, N'sasiphos®, plant extracts and excipients were supplied from the manufacturer (CRMTA, Kinshasa, DRC).

2.2. Materials

Analyzes were performed using an Agilent / HP1100 series HPLC chain, HP series thermostatically controlled compartments (G1316A)

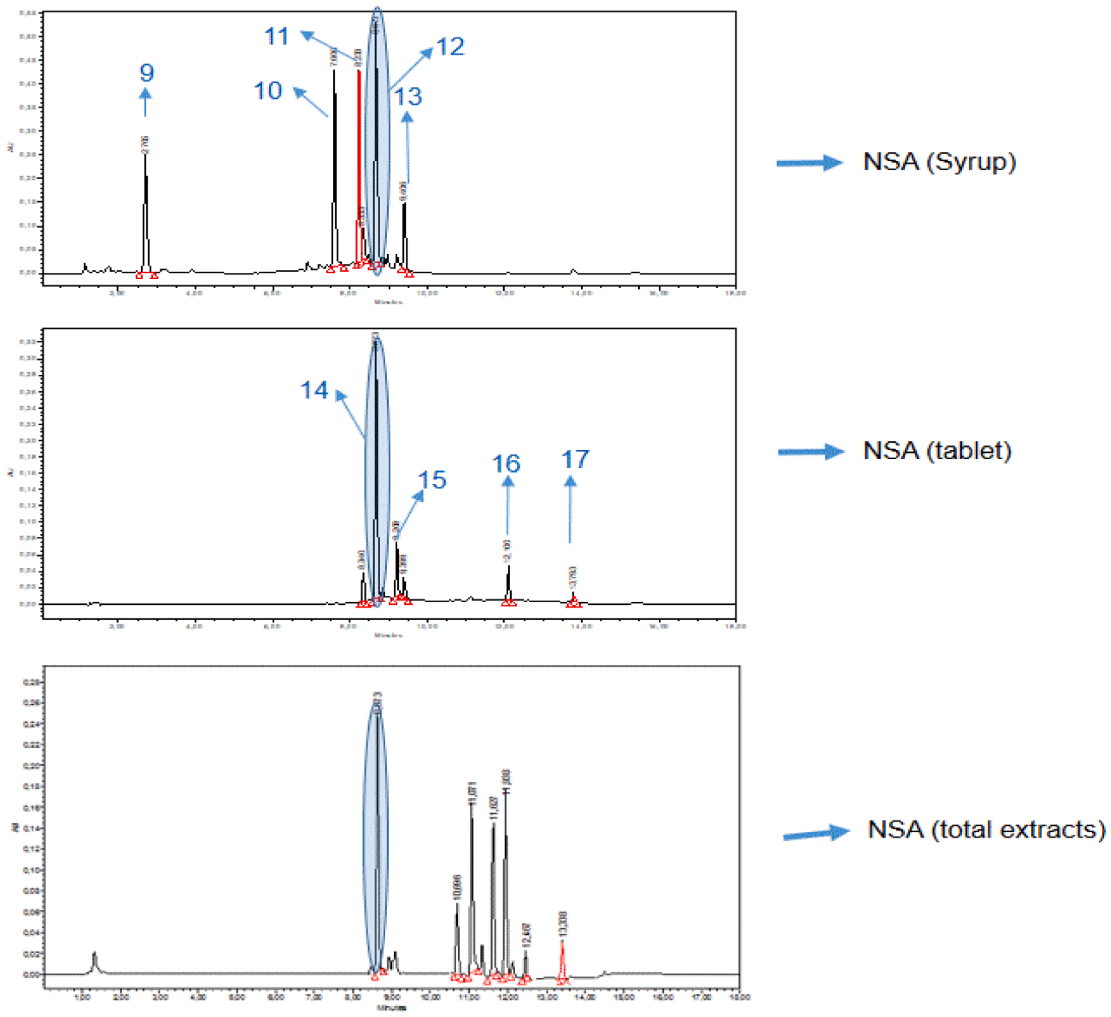

Fig. 4. Nsansiphos (syrup, tablets and total extracts) chromatograms.

equipped with an Agilent quaternary pump (G1311A) and an HP diode array detector (G1315A), with an Agilent degasser (G1379A) Series JP13212087 and an Agilent automatic injector (G1313A), controlled by Chromaster software (Antwerp, Belgium). Two chromatographic columns were used, an XBridge C18 (250 × 4.6 mm i.d.; 5 µm particle size) and an XBridge C18 (100 × 4.6 mm i.d.; 3.5 µm particle size) both from Waters (Milford, MA, USA). A GRAM FV-220C electronic balance provided by IPESAGE SAS, France and an IKA® C-MAG MS4 stirrer, provided by Grosseron SAS, France. The flow rate of the mobile phase was set at 1.0 mL / min and the detection was carried out at 220 nm, 254 nm, 280 nm and 320 nm. Gradient transfer from long to short column was performed using HPLC calculator V3.0 software (University of Geneva, Geneva, Switzerland). The accuracy profiles as well as the statistical calculations including the validation results were obtained using e-noval® V3.0 software (Pharmalex, Mont Saint Guibert, Belgium). The stability apparatus used was from the brand of Weiss Technik (Paris, France) of size Pharma 2000. The samples were stored for 6 months in this chamber at a temperature of 40 ± 2 °C and the relative humidity of 75 ± 5% (add reference = ICH Q1A (R2)).

2.3. Standard sample preparation

Collected samples had following dosages:

- Ø MEY sirop: each milliliter (1 ml) contained 50 mg of *Hymenocardia acida* Tannins.
- Ø MEY tablet: each tablet contained 500 mg of *Hymenocardia acida* Tannins.
- Ø NSA: each 5 ml contain 60 mg of *Cruciaceae* total extract including Alcaloids, triterpenic steroids, catechic tannins, flavonoids and anthocians.
- Ø DIA: each tablet contains 30 mg of extract including Alcaloids, tannins, steroids and *rubiaceae* triterpenoids.
- Ø NZI tablet: each tablet contained 100 mg of capsaicin derivatives from total extract of zingiberaceae.

2.3.1. Solution used for calibration and validation

The stocks solutions of DIA and MEY were prepared by dissolving 5,0 g of each material in 20 mL methanol; however, NSA et NZI samples were prepared by dissolving 3,0g and 10,0 g, respectively in 50 ml methanol.

For the calibration standards (CS), dilutions were performed in methanol–water (50:50, v/v) in order to obtain solutions at concentrations of 25mg/mL, 50mg/mL and 75mg/mL for DIA and MEY, except for NSA the concentrations were 6mg/mL, 12mg/mL and 18mg/mL, and for NZI the concentrations were 50mg/mL, 100mg/mL and 150mg/mL

Three concentration levels (50%, 100% and 150%) were sufficient to generate different regression models for the calibration.

For validation standards (VS), independent stock solutions of NSA, DIA, NZI, MEY were prepared in the same way as described for the CS. For the matrix, the excipients were added in the preparation according

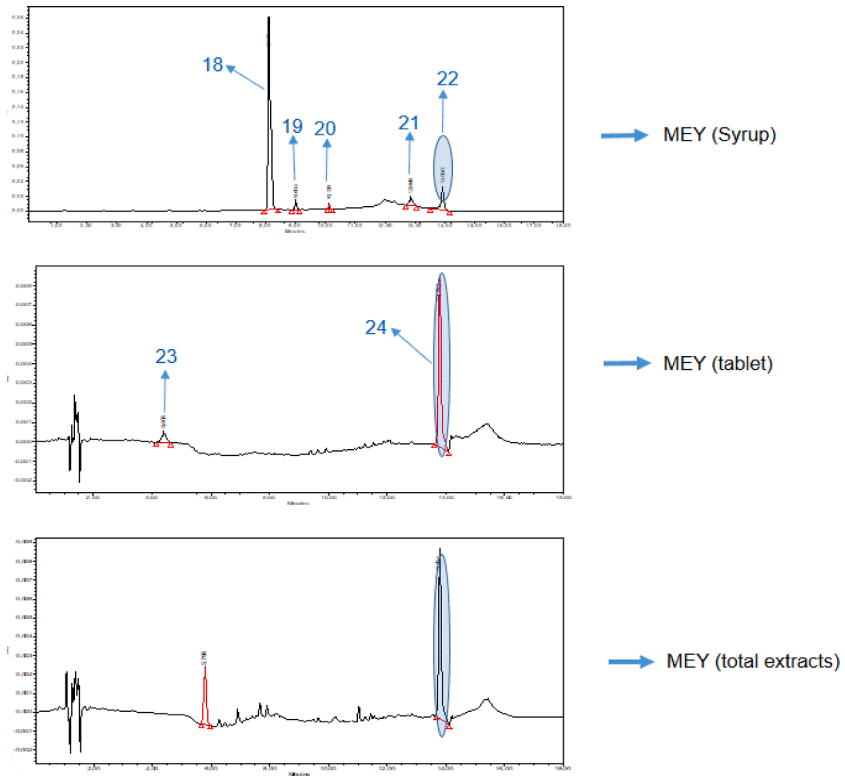

Fig. 5. Meyamycin (syrup, tablets and total extracts) chromatograms.

Table 3
Retention time (RT, minutes) before and after geometric transfer (using compounds 3, 4, 12, 14, 22 et 24 as biomarkers).

Products	Compound	Initial method Retention time	Relative retention time	Transfered method Retention time	Relative retention time	Gap (minutes)
NZI tablets	Compound 1	24.061	0.778	2.643	0.251	0.527
	Compound 2	26.524	0.858	3.425	0.325	0.533
	Compound 3	28.308	0.915	9.470	0.899	0.016
	Compound 4	30.926	1.000	10.537	1.000	0.000
DIA tablets	Compound 5	20.873	0.615	9.518	0.793	0.178
	Compound 6	24.379	0.719	10.694	0.894	0.175
	Compound 7	27.866	0.821	11.012	0.920	0.099
	Compound 8	33.928	1.000	11.964	1.000	0.000
NSA Surup	Compound 9	7.893	0.400	2.705	0.288	0.112
	Compound 10	14.441	0.732	7.600	0.808	0.076
	Compound 11	16.341	0.828	8.233	0.875	0.047
	Compound 12	16.911	0.857	8.333	0.886	0.029
	Compound 13	18.231	0.924	8.637	0.918	0.006
NSA tablets	Compound 14	16.179	0.588	8.340	0.605	0.017
	Compound 15	18.239	0.663	8.673	0.629	0.034
	Compound 16	16.902	0.614	9.208	0.668	0.054
	Compound 17	27.528	1.000	13.793	1.000	0.000
MEY syrup	Compound 18	12.468	0.368	8.116	0.583	0.215
	Compound 19	15.451	0.456	9.004	0.646	0.190
	Compound 20	24.964	0.736	10.129	0.727	0.009
	Compound 21	28.271	0.834	12.889	0.925	0.091
	Compound 22	33.896	1.000	13.930	1.000	0.000
MEY tablets	Compound 23	13.603	0.401	4.404	0.319	0.082
	Compound 24	33.925	1.000	13.792	1.000	0.000

to the formulation of each product.

Subsequent dilutions in methanol–water (50:50, v/v) were carried out in order to obtain 5 solutions at different concentration levels (50%, 75%, 100%, 125% and 150%). 25mg/mL, 37,5mg/mL, 50mg/mL, 62,5mg/mL and 75g/mL of DIA and MEY. For NSA 6mg/mL, 9mg/mL, 12mg/mL, 15mg/mL and 18mg/mL, then NZI 50mg/mL, 75mg/mL, 100mg/mL, 125mg/mL and 150mg/mL). The five concentrations levels for the validation standards (50%, 75%, 100%, 125% and 150%) were independently prepared in the matrix, simulating the formulation as much as possible and its future routine analysis. They were selected to evaluate the method ability at concentration of counterfeit products.

2.3.2. *Samples for analysis*

The solutions for analysis were prepared with the methanol-water (50-50, v/v) mixture to obtain a target concentration of 50mg/mL for DIA and MEY, of 12mg/mL for NSA and 100mg/mL for NZI.

Table 4
Summary of the validation criteria.

Validation criteria	Conc (mg/ml)	NZI (Tablet)	Conc (mg/ml)	NSA (Syrup)	NSA (Tablet)	Conc (mg/ml)	DIA (tablet)	MEY (Syrup)	MEY (Tablet)
Trueness: Absolute bias (mg/mL) (relative bias (%))	50	1.31 (0,66)	0.2	0.01 (0.23)	0.02 (0.62)	25.0	0.16 (0.62)	0.34 (1.37)	0.37 (1.43)
	75	0.62 (0,82)	0.3	0.01 (0.83)	0.01 (1.05)	37.5	0.15 (0.38)	0.52 (1.39)	0.63 (1.68)
	100	0.47 (0,47)	0.4	0.04 (1.67)	0.03 (1.41)	50.0	1.00 (2.10)	0.75 (1.46)	0.95 (1.89)
	125	1.88 (2,35)	0.5	0.02 (-0.29)	0.04 (3.08)	62.5	0.53 (1.33)	0.16 (0.29)	0.48 (0.76)
	150	0.50 (0,75)	0.6	0.01 (-0.30)	0.01 (-1.89)	75.0	1.88 (2.51)	0.01 (0.04)	0.21 (0.26)
Precision: Repeatability (%)/Intermediate precision (%)	50	1.66 /1,78	0.2	3.09/3.82	1.40/2.30	25.0	0.78/1.16	3.17/3.23	1.64/1.68
	75	1.89 /1,92	0.3	1.63/2.52	0.96 / 1.66	37.5	2.61/2.72	2.28 / 2.37	1.62 / 1.75
	100	1.37 /1,97	0.4	1.62/2.41	1.50 / 1.56	50.0	2.54/2.56	2.00/ 2.12	1.66 / 1.84
	125	1.76 /2,14	0.5	1.46/1.59	1.64 / 1.93	62.5	1.74/2.07	1.48 / 1.98	1.45 / 1.61
	150	2.16 /2,76	0.6	1.43/1.99	1.20/1.60	75.0	1.20/1.32	1.76 / 1.92	1.85 /1.97
Linearity:	Slope	1.001	Slope	1.027	0.980	Slope	0.980	0.991	0.990
	Intercept	0.203	Intercept	0.008	0.013	Intercept	0.932	0.788	0.931
	R^2	0.999	R^2	0.998	0.994	R^2	0.994	0.997	0.998

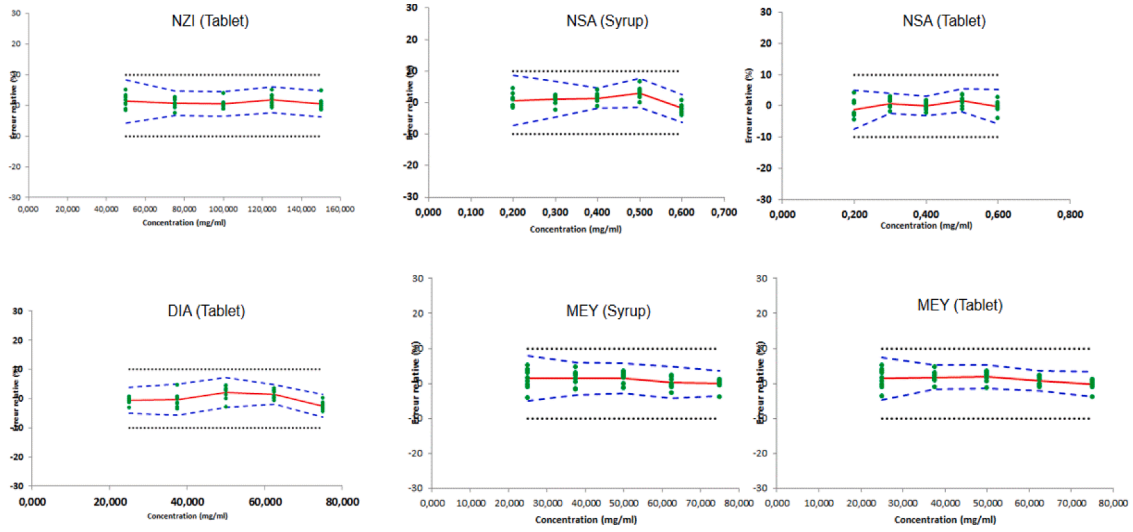

Fig. 6. Method accuracy profile for Zinginalis (tablets), Nsansiphos (syrup and tablets), Diazostimule (tablets) and Meyamycin (syrup and tablet).

3. Results and discussion

Fig. 1 presents the images of marketed herbal medicines used in this study and the Table 1 illustrates the different batch.

3.1. Chromatographic fingerprint

ITMs fingerprint establishment was performed based on the chromatographic conditions used by Tshisekedi et al. shown in Table 2. The long chromatographic column was used in the first place to increase the chromatographic peaks resolution. Next, the successful geometric transfer was achieved using the short column in order to reduce the analysis time and minimize the amount of organic solvent needed for elution (Guillarme et al., 2007, 2008, Mbinze et al., 2012, 2015). The elution gradient used for the two chromatographic columns are depicted in Table 2.

The samples as well as the active ingredients (total extract) were used under these conditions of gradients elution. The obtained chromatograms are shown in Figs. 2–5

The chromatographic peaks obtained were well separated in all cases, whether we used long column (data not shown) or short column. Four wavelengths were tested for detection, of which only 280 nm was adopted as the optimal value because of a larger number of chromatographic peaks were observed.

Other chromatographic peaks were observed in finished products chromatograms while they were not visible in active ingredients chromatograms (total extracts). These chromatographic peaks could be attribute to conservators or excipients. A biomarker was set in each case, that is to say a product that is found in the active ingredients (total extract) and in finished product at a remarkable intensity. The purity of these biomarkers was determined by DAD detector following the spectral comparison at different points.

The use of short column allowed to reduce the consumption of organic solvent and the analysis time was cut down 3-fold shorter (from 60 min to 18 min), observation already demonstrated in other studies (Mbinze et al., 2012, 2015).

Geometric transfer quality is confirmed by comparing relative retention time which is calculated from the last peak as demonstrated in our previous work (Mbinze, 2012, 2015). Table 3 shows the comparison of the relative retention times before and after the transfer of the

Table 5
Accelerated stability study results (identification and quantification of biomarkers).

Drugs	Form	Batches	Exp date	First Month		Third Month		Sixth Month	
				RT (min)	Percentage	RT (min)	Percentage	RT (min)	Percentage
DIA	Tablets	DZC1	02/2022	9.5	95.4 ± 2.1	9.5	94.7 ± 1.2	9.5	93.2 ± 1.6
		DXC3	03/2023	9.5	88.1 ± 2.0	9.5	86.5 ± 1.8	9.5	85.1 ± 1.7
		DZC3	10/2022	9.5	103.3 ± 2.4	9.5	100.9 ± 2.1	9.5	100.1 ± 2.4
		DZC5	05/2023	9.5	83.4 ± 2.4	9.5	83.1 ± 1.9	9.5	82.1 ± 2.7
MEY	Tablets	MLC5	03/2022	13.9	84.3 ± 2.7	13.9	82.7 ± 2.4	13.9	81.6 ± 2.7
		MXC7	04/2024	13.9	108.1 ± 2.4	13.9	105.4 ± 2.1	13.9	105.0 ± 2.0
		MWC7	06/2024	13.9	97.5 ± 1.7	13.9	95.7 ± 2.5	13.9	94.5 ± 1.9
		MYC8	08/2024	13.9	90.5 ± 2.4	13.9	88.7 ± 2.6	13.9	87.4 ± 1.2
	Syrup	MLS1	02/2022	13.9	98.1 ± 1.1	13.9	96.8 ± 1.2	13.9	95.4 ± 0.7
		MXS1	10/2023	13.9	94.3 ± 1.3	13.9	93.7 ± 2.0	13.9	92.8 ± 0.9
		MYS3	05/2024	13.9	108.1 ± 0.7	13.9	106.1 ± 1.5	13.9	105.8 ± 1.4
		MWS4	06/2024	13.9	93.7 ± 1.4	13.9	92.0 ± 0.8	13.9	90.9 ± 0.8
NZI	Tablets	ZYC1	02/2022	9.4	94.2 ± 1.8	9.4	93.0 ± 1.8	9.4	90.9 ± 2.1
		ZZC1	10/2021	9.4	90.1 ± 1.5	9.4	88.7 ± 2.0	9.4	86.7 ± 2.0
		ZZC2	11/2022	9.4	102.7 ± 1.5	9.4	101.7 ± 2.1	9.4	99.2 ± 1.8
		ZYC2	07/2023	9.4	87.4 ± 2.7	9.4	86.0 ± 1.9	9.4	85.4 ± 1.6
NSA	Tablets	NZC1	02/2022	8.4	85.7 ± 1.7	8.4	83.1 ± 1.8	8.4	81.4 ± 2.4
		NZC2	07/2022	8.4	104.4 ± 2.1	8.4	102.9 ± 1.9	8.4	100.4 ± 2.1
		NZC3	04/2023	8.4	95.7 ± 2.4	8.4	94.2 ± 2.4	8.4	93.0 ± 1.9
		NZC3	05/2023	8.4	91.4 ± 1.9	8.4	89.7 ± 2.1	8.4	89.1 ± 2.4
	Syrup	NZS1	05/2022	8.4	94.7 ± 1.0	8.4	93.6 ± 1.4	8.4	90.1 ± 1.2
		NZS2	01/2023	8.4	108.8 ± 1.1	8.4	107.1 ± 1.6	8.4	105.5 ± 1.0
		NZS3	11/2023	8.4	87.4 ± 0.7	8.4	86.4 ± 1.0	8.4	84.3 ± 0.9
		NZS4	05/2023	8.4	105.6 ± 0.9	8.4	103.77 ± 1.7	8.4	102.8 ± 0.8

method, and shows that the two data sets are in close proximity (the difference being less than one minute), which suggests that the geometric transfer is reliable.

This makes it possible to validate the geometric transfer. The chromatographic conditions retained for this generic method are as follow: the temperature of the oven (30 °C), the wavelength of 280 nm, and flow rate of the mobile phase in gradient mode of 1.0mL / min.

3.2. Method validation

The method using the short column was validated using the total extracts as references. The strategy of total error was used to decide on the reliability of the method according to the international guideline recommendations (Mbinze et al., 2013, Mboni et al.,2014]. The acceptance limit was set at ±10% (Bouabidi et al., 2017, Boulanger et al., 2009).

Table 4 presents the results of the accuracy, precision and linearity of the developed generic method. It is fair with the relative and absolute biases of less than 10%. It is precise with good repeatability and intermediate precision because the variation coefficients are less than 10% at all concentration levels. The method is linear at the level of concentrations studied with the coefficients of determination close to 1, which shows a good agreement between the theoretical and practical concentrations. Fig. 6 illustrates the different accuracy profiles of this developed method, confirming the accuracy of the method because all beta tolerance intervals are within the acceptance limits set at 10%. The lower and upper limits of quantification are determined by the intercession between the acceptance limits and the beta tolerance intervals, are the lower and upper concentrations, respectively.

The developed method exhibits good selectivity because the extra peaks generated by the finished products did not interfere with biomarker chromatographic peaks in all cases (data not shown). This good resolution indicates that the developed HPLC-DAD generic method is also selective and can be used for determination of ITMs biomarkers in the presence of its extra peaks.

The generic method has therefore been successfully validated according to the total error strategy, which demonstrated the ability of analyzing these six different ITMs without changing the chromatographic conditions.

3.3. Quality control of samples

The stability test was carried out on 4 commercial batches of each drug (i.e. 24 analyzed samples). The results of biomarker identification and quantification using the validated generic method are shown in Table 5

The results obtained showed that all the 6 drugs were chemically stable during the period of this study because the retention times remained constant and the percentages of biomarkers relative to the active ingredients were not markedly modified.

4. Conclusion

The main objective of this work was to develop a generic method of identification and quantification of six Improved Traditional herbal Medicines manufactured and marketed in DRC with the goal of enhancing local production and guaranteeing the quality of herbal drug products. Indeed, the herbal medicines do not have the chemical control methods available for the pharmaceutical quality control laboratory in DRC. To achieve this, we used High Performance Liquid Chromatography, a separative analytical technique to develop the generic method capable of analyzing the six herbal drugs without changing the chromatographic conditions. Subsequently, the developed generic method was validated according to the total error strategy and showed good precision, trueness, accuracy and linearity. The proposed method can be used by the National Quality Control Laboratory of the DRC for the analysis of real herbal drug samples. With this in mind, an accelerated stability study was carried out on 24 samples (batches) for six months. The results obtained show that the products remained chemically stable (retention time and percentages of biomarkers were unchanged) over time of analysis. This generic method represents a major breakthrough in ensuring the chemical quality of improved traditional herbal medicines in general and of these six medicines in particular, and to fight against the circulation of counterfeit medicines in the DRC.

Author agreement

All the authors agree with submitting this research article in Phytomedicine Plus. And no conflict is noticed regarding our paper.

Every single person involved in this work as author is aware of every

stage of this submission and accepted its submission in this editorial system.

Declaration of Competing Interest

This work is an original research from the drug analysis unit of the faculty of pharmaceutical sciences of the University of Kinshasa in D.R. Congo and the Centre for Research on Improved Traditional Medicines. All data presented in this paper are the results of laboratory experiments and there is not conflict of interest related to them.

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References

Basimine, C.G., Cizungu, C.N., Mubalama, M.J., Mugumaarhahama, Y., Mushamalirwa, G., Karume, K., Nachigera, M.G., Schmitzd, S., 2021. Suitability for agroforestry implementation around Itombwe Natural Reserve (RNI), eastern DR Congo: application of the analytical hierarchy Process (AHP) approach in geographic information system tool. Trees For. People 6, 100125.

Boulanger, B., Rozet, E., Moonen, F., Rudaz, S., Hubert, Ph., 2009. A risk-based analysis of the AAPS conference report on quantitative bioanalytical methods validation and implementation. J. Chromatogr. B 877, 2235–2243.

Habyalimana, V., Mbinze, J.K., Yemoa, A.L., Waffo, C., Diallo, T., Kalenda, T.N., Kadima Ntokamunda, J-L., Lebrun, P., Hubert, Ph., Marini, R.D., 2017. Application of design space optimization strategy to the 1 development of lc methods for simultaneous analysis of 18 antiretroviral medicines and 4 major excipients used in various pharmaceutical formulation. J. Pharm. Biomed. Anal. 139, 8–21.

Hugéab, J., de Bisthoven, L.J., Mushiete, M., Rochette, A.J., Candido, S., Keunen, H., Dahdouh, G.F., Koedam, N., Maarten, P.M., 2020. EIA-driven biodiversity mainstreaming in development cooperation: confronting expectations and practice in the DR Congo. Environ. Sci. Policy 104, 107–120.

Guillarme, D., Nguyen, D., Rudaz, S., Veuthey, J.-L., 2007. Method transfer for fast liq uid chromatography in pharmaceutical analysis: application to short columns packed with small particle. Part I: isocratic separation. Eur. J. Pharm. Biopharm. 66, 475–482.

Guillarme, D., Nguyen, D., Rudaz, S., Veuthey, J.L., 2008. Method transfer for fast liq uid chromatography in pharmaceutical analysis: application to short columns packed with small particle. Part II: gradient experiments. Eur. J. Pharm. Biopharm. 68, 430–440.

Mbinze, J.K, Dispas, A., Lebrun, P., Mavar, T.J., Habyalimana, V., Kalenda, N., Rozet, E., Hubert, Ph., Marini, R.D, 2013. Application of an innovative design space optimization strategy to the development of LC methods for the simultaneous screening of antibiotics to combat poor quality medicines. J. Pharm. Biomed. Anal. 85, 83–92.

Mbinze, J.K., Lebrun, P., Debrus, B., Dispas, A., Kalenda, N., Mavar, T.J., Schofield, T., Boulanger, B., Rozet, E., Hubert, Ph., Marini, R.D, 2012. Application of an innovative design space optimization strategy to the development of liquid chromatographic methods to combat potentially counterfeit nonsteroidal anti inflammatory drugs. J. Chromatogr. A 1263, 112–113.

Mbinze, JK, Yemoa, A., Lebrun, P., Sacré, P.-Y., Habyalimana, V., Kalenda, N., Bigot, A., Atindehou, E., Hubert, Ph., Marini, R.D., 2015. Fighting poor quality medicines: development, transfer and validation of generic HPLC methods for analyzing two WHO recommended antimalarial tablets. Am. J. Anal. Chem. 6, 127–144.

Mboni, H.M., Keymeulen, F., Ngezahayo, J., Bakari, A.S., Mutombo, E.K., Kahumba, B.J., Duez, P., Stévigny, C., Lumbu, J.B., 2020. Antimalarial herbal remedies of Bukavu and Uvira areas in DR Congo: An ethnobotanical survey. J. Ethnopharmacol. 249, 112422.

Ngoy, M.S., Faucon, M.P., Kamengwa, K.C., Colinet, G., Mahy, G., Luhembwe, M.N., Meerts, V.P., 2015. Three years of phytostabilisation experiment of bare acidic soil extremely contaminated by copper smelting using plant biodiversity of metal-rich soils in tropical Africa (Katanga, DR Congo). Ecol. Eng. 82, 81–90.

Tshisekedi, T.P., Mutwale, K.P., Kabongo, K M.J., Kabika, LH., Kabamba, NN., Dibungi, KT., Jansen, O., Wauters, J.N., Tits, M., Angenot, L., Rozet, E., Hubert, Ph., Marini, R.D., Frédérich, M, 2016. Fingerprinting and validation of a LC-DAD method for the analysis of biflavanones in Garcinia kola-based antimalarial improved traditional medicines. J. Pharm. Biomed. Anal. 128, 382–390.