



Physicochemical and chromatographic method of characterization of *Matricaria recutita* tinctures

[Método de caracterización fisicoquímica y cromatográfica de tinturas de *Matricaria recutita*]

Jonathan Parra¹, Pedro M. García-Barrantes¹, Gerardo Rodríguez², Beatriz Badilla¹

¹Instituto de Investigaciones Farmacéuticas (INIFAR), Universidad de Costa Rica, San Pedro, San José, Costa Rica.

²Laboratorio de Fitoquímica, Escuela de Química Industrial, Universidad Nacional de Costa Rica, Heredia, Costa Rica.

*E-mail: jonathan.parra@ucr.ac.cr

Abstract

Context: The pharmacological activity of medicinal products containing plant materials depends on their specific components. However, these components are not characterized in their entirety in all cases. Therefore, manufacturing processes must be duly characterized and validated.

Aims: To characterize a chamomile (*Matricaria recutita*) tincture through chemometric analysis of chromatographic data in order to establish quality parameters for its production.

Methods: Various chamomile tinctures were manufactured and the precision and robustness of the production process for each was verified. The physicochemical properties of the tinctures were characterized and their chromatographic digital fingerprints analysed through chemometric methods.

Results: A good correlation between the physicochemical characterization and the chromatographic analysis was demonstrated. The preparation methodology was proved to be repeatable as long as the source of the plant material is not altered.

Conclusions: The principal component multivariate analysis of chromatograms was a helpful and simple tool for the characterization and traceability of the production method.

Keywords: Liquid chromatography; *Matricaria recutita*; principal components analysis; tincture.

Resumen

Contexto: Para los productos medicinales que contienen material vegetal, la actividad farmacológica depende de sus componentes específicos; sin embargo, estos componentes no se encuentran completamente caracterizados en todos los casos; por ello, los procesos de manufactura deben ser caracterizados y validados.

Objetivos: Caracterizar una tintura de manzanilla (*Matricaria recutita*) por medio del análisis quimiométrico de datos cromatográficos para establecer parámetros de calidad en su producción.

Métodos: Se elaboraron varias tinturas de manzanilla, verificando la precisión y robustez del proceso de producción. Se caracterizó las propiedades fisicoquímicas de las tinturas y se analizó la huella digital cromatográfica por métodos quimiométricos.

Resultados: Se demostró una buena correlación entre la caracterización fisicoquímica y el análisis cromatográfico. El método de preparación demostró ser repetible si no se altera la fuente del material vegetal.

Conclusiones: El análisis multivariable por componentes principales de los cromatogramas demostró ser una herramienta útil y sencilla para la caracterización y trazabilidad del método de preparación.

Palabras Clave: Análisis por componentes principales; cromatografía líquida; *Matricaria recutita*; tintura.

ARTICLE INFO

Received | Recibido: October 5, 2015.

Received in revised form | Recibido en forma corregida: December 28, 2015.

Accepted | Aceptado: December 9, 2015.

Available Online | Publicado en Línea: January 18, 2016.

Declaration of interests | Declaración de Intereses: The authors declare no conflict of interest.

Funding | Financiación: This work was supported by a grant of Consejo Nacional de Rectores (CONARE, CNR-351-08) of Costa Rica.

Academic Editor | Editor Académico: Gabino Garrido.

INTRODUCTION

During the XIX century, pharmacists developed and characterized different manufacturing processes for plant materials including decoctions, infusions, powders and tinctures. These techniques are still currently used to process herbs and spices for culinary and medicinal purposes. Tinctures are hydroalcoholic herbal preparations that maintain their potency for a longer period of time than infusions or decoctions (Lushear, 2005). Tincture is defined by USP in its general chapter about Botanical Extracts as a “liquid preparation obtained through the extraction of plant material with alcohol or hydroalcoholic mixtures”. Traditionally, tinctures possess a 10 g concentration of plant material per 100 mL of preparation and are prepared from coarse powder or fine cuts of plant materials through percolation or maceration techniques (USP, 2014).

The World Health Organization’s report on the World Medicines Situation 2011 states on its traditional medicine section (WHO/EMP/MIE-/2011.2.3) that the latter (including herbal medicine) is used in all countries of the world and specifically in developing countries it is used by 70% and 95% of the population as primary healthcare. The global market for traditional medicine was estimated at 83 billion dollars in 2008 (WHO, 2011).

The pharmacological activity of medicinal products containing plant material with pharmacological properties depends on their specific components. However, these components are not yet characterized in all cases. Thus, despite possessing beneficial effects, they may become a source of adverse reactions and interactions with other medicinal products if the manufacturing processes are not characterized and validated (Pribitkin, 2005). In order to ensure the safety and integrity of these products is necessary the authentication of the starting plant material; chemometric techniques, an extension of phytochemical fingerprinting, are the utilization of statistical evaluation tools such as principal component analysis (PCA) to evaluate either a broad-range spectroscopic scan or a representative chromatographic segment of a given sample as compared to a compiled population of

authenticated reference samples (Khan and Smillie, 2012).

Chamomile (*Matricaria recutita* L.) is one of the oldest medicinal herbs known to human kind. Chamomile preparations are commonly used for many human ailments (Srivastava et al., 2010).

This research aims to establish a tool to analyze chromatographic data from the preparation of *Matricaria recutita* tinctures from different suppliers in order to characterize its preparations and to establish quality parameters for its production.

MATERIAL AND METHODS

Plant material

Dried floral capitulum of *Matricaria recutita* was provided by two different national suppliers, the plant material of both suppliers was cultivated in Cartago, Costa Rica. The plant material was identified through the pharmacopeial identification test with *p*-dimethylaminobenzaldehyde and analyzed using the pharmacopeial tests of humidity, total ash and microbiological limits (USP, 2014).

Tincture preparation

Tinctures were prepared pursuant to the methodology established in the general chapter <565> on Botanical Extracts of USP 37. The necessary quantities were used to obtain a final concentration of 10 g of plant material per 100 mL of tincture.

Plant material was mixed with a sufficient amount of 50% ethanol in order to be soaked uniformly and completely and was left to stand for 15 minutes. The moist sample was placed in an 800 mL plastic percolator and compacted. Ethanol 50% (500 mL) was added. As soon as the liquid was about to drip, the bottom orifice was closed and was left in maceration for 24 hours. Subsequently, percolation was performed slowly, at a rate no higher than 1 mL/minute. Percolation was sustained, gradually adding small quantities of 50% ethanol until a 500 mL volume of tincture was obtained in the collection container. The latter was shaken and stored under refrigeration (2 – 8 °C) protected from light in order to avoid decomposition or solvent loss (USP, 2014).

Three production batches were produced to verify the precision of the manufacturing process. The manufacturing process was replicated three times per batch. Two more batches were produced using different suppliers of plant material in order to verify the robustness of the manufacturing process. The manufacturing scheme is shown in Fig. 1.

Physicochemical characterization

Relative density, alcohol grade, refraction index, pH and dry residue pharmacopeial tests were performed. The measurements of the apparent alcoholic grade and relative density were performed using classic analytical methods by immersing an alcoholmeter in a test tube with the tincture and a pycnometer, respectively. A refractometer and pH meter were used to measure the refraction index and pH, respectively (Padró and López, 2000; BP, 2008).

In order to measure the dry residues, 1 g of tincture was placed in a porcelain capsule with a known weight and evaporated to dryness in an oven at 105°C and dried to constant weight. The dry residue was determined by the difference.

Chromatographic characterization

The chromatographic digital fingerprint of the tincture was obtained through High Performance

Liquid Chromatography on Shimadzu equipment with an SPD-M20A diode array detector coupled to a LC-20AT quaternary pump. The latter uses a 250 mm x 4.6 mm C18 Phenomenex column with 5 µm granulometry. The solvent system used is water acidified with 0.01% trifluoroacetic acid and methanol. A concentration gradient at a rate of 62.5:37.5 from 0 to 20 minutes, 60:40 from 20 to 40 minutes and 0:100 from 40 to 50 minutes in water and methanol, respectively, was used. A flow of 1 mL/min. was used. Samples were used at room temperature. The detection wavelength was 335 nm.

Statistical analysis

Measures of central tendency, standard deviation and confidence intervals were calculated in the measurement of the tincture's physicochemical properties. Furthermore, an ANOVA data analysis and a Tukey's post hoc test analysis were performed using IBM SPSS Statistics 19 (IBM Company, USA).

Chromatographic data was analyzed using principal component analysis with the aid of The Unscrambler program 10.1 (CAMO Software, Norway).

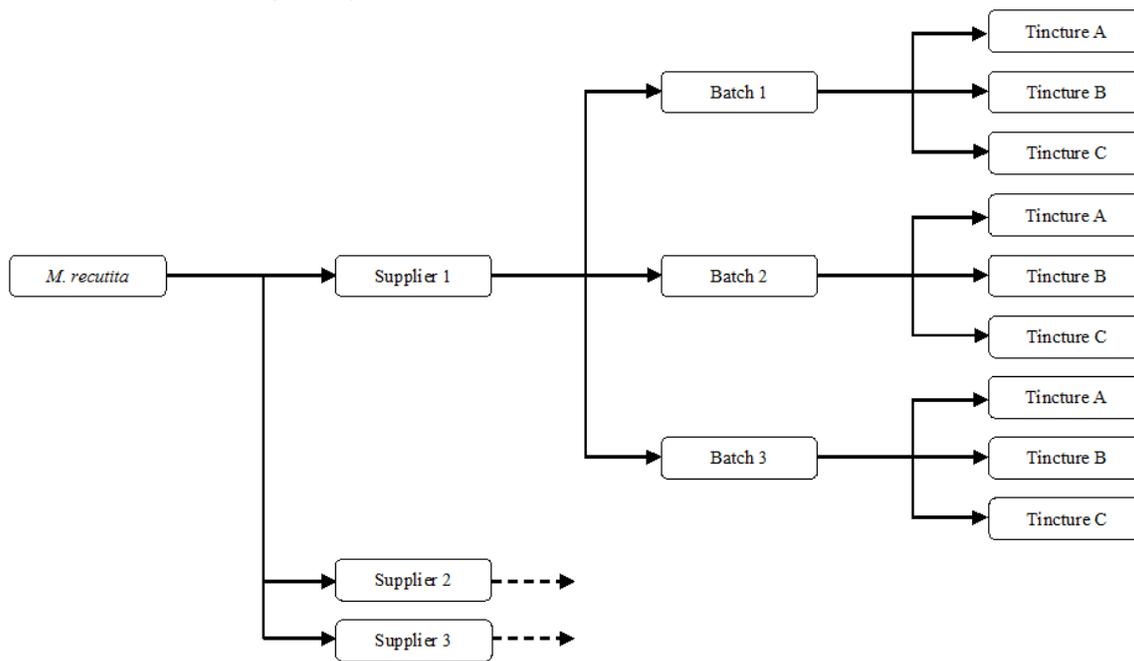


Figure 1. Tincture's manufacturing scheme.

RESULTS AND DISCUSSION

Data on the characterization of the physicochemical properties of the manufactured tincture is shown on Table 1.

Table 1. Physicochemical characterization of the *Matricaria recutita* tincture.

Characteristic	Average \pm S.D.	Lower limit	Upper limit
Alcoholic grade ($^{\circ}$)	47.5 \pm 0.3	46.9	48.2
pH	6.74 \pm 0.05	6.63	6.84
Refraction index	1.3612 \pm 0.0001	1.3610	1.3613
Relative density	0.9406 \pm 0.0008	0.9388	0.9424
Percentage of solids (%)	1.38 \pm 0.05	1.28	1.48

The change in the alcoholic grade is within the limits allowed by USP. The latter allows variation coefficient values of up to 5% (USP, 2014). pH variations in the tincture can be associated with the variations in the alcoholic grade, mainly due to the ethanol evaporation (Siries et al., 2009). In both cases, differences are within the expected margin.

Measurements of the refraction index demonstrated significant differences between the tinctures ($p = 0.02$). However, despite the fact that the differences are significant, they are within the uncertainty levels of the apparatus and they are less than those found in similar studies on the quality evaluation of *Pedillanthus tithymaloides* tinctures, which indicates that the differences are acceptable. (Padró and López, 2000; Padró and Marín, 2008 a;b).

The refraction index is directly related to the total solid content and therefore with the preparation density, due to the fact that they are all indicators of the quantity and nature of the particles extracted from the plant material (Carmona et al., 2009). In a study performed using *Matricaria recutita*, different aqueous extracts were compared using the refraction index, solid residues and relative density as extraction effectiveness parameters (Barene et al., 2003).

Measures of solid residues demonstrated significant differences ($p = 0.006$). However, Tukey's test demonstrated that the tinctures prepared with plant material number two had a different distribution whilst the tinctures prepared with the same plant material had a similar distribution. Relative density measurement results demonstrated a behavior similar to that shown by the solid residues measurements; however, in this case, the differences were obtained for both plant material numbers two and three. In short, it was demonstrated that plant material is the variable affecting the repeatability of the tincture's manufacturing process because despite possessing similar refraction indices, each batch of plant material generated tinctures with different total solid contents and density.

The chromatographic digital fingerprint is recommended for the identification and analysis of complex pharmaceutical systems. Furthermore, its use has been recommended to verify the identity and to control the quality of natural products with medicinal qualities. In general, there are several registered chromatographic peaks in a given herbal extract due to the complexity of products of botanical origin. Therefore, an integral analysis of the chromatographic graph allows the comparison of the different extracts in terms of the concentration and nature of extracted substances (Gan and Ye, 2006; Gong et al., 2006). The graph results of the chromatographic data are shown in Fig. 2.

Principal component analysis is used to statistically analyze the differences in the chromatograms. Principal component analysis provides a graphical representation of the relationship between variables and samples, which in turn allows to determine the extent to which a variable causes the samples to be similar or not. The use of this type of analysis is recommended to evaluate the quality consistency of herbal products, allowing a classification by sample and an analysis of their similarity based on the chromatographic digital fingerprints. Therefore, the latter is a good tool for quality control of herbal medicines (Xie et al., 2008). Fig. 3 shows the sample distribution according to the principal component analysis.

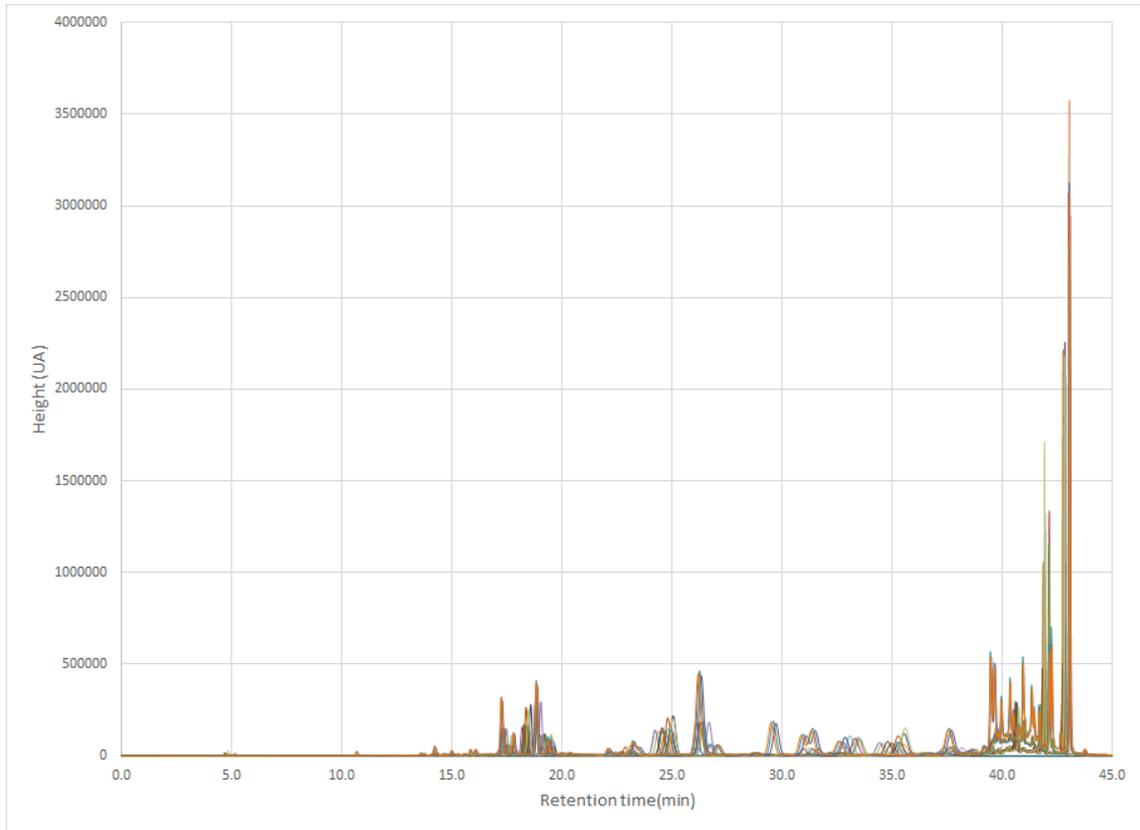


Figure 2. Superposition of the chromatograms for each batch of *Matricaria recutita* tincture.

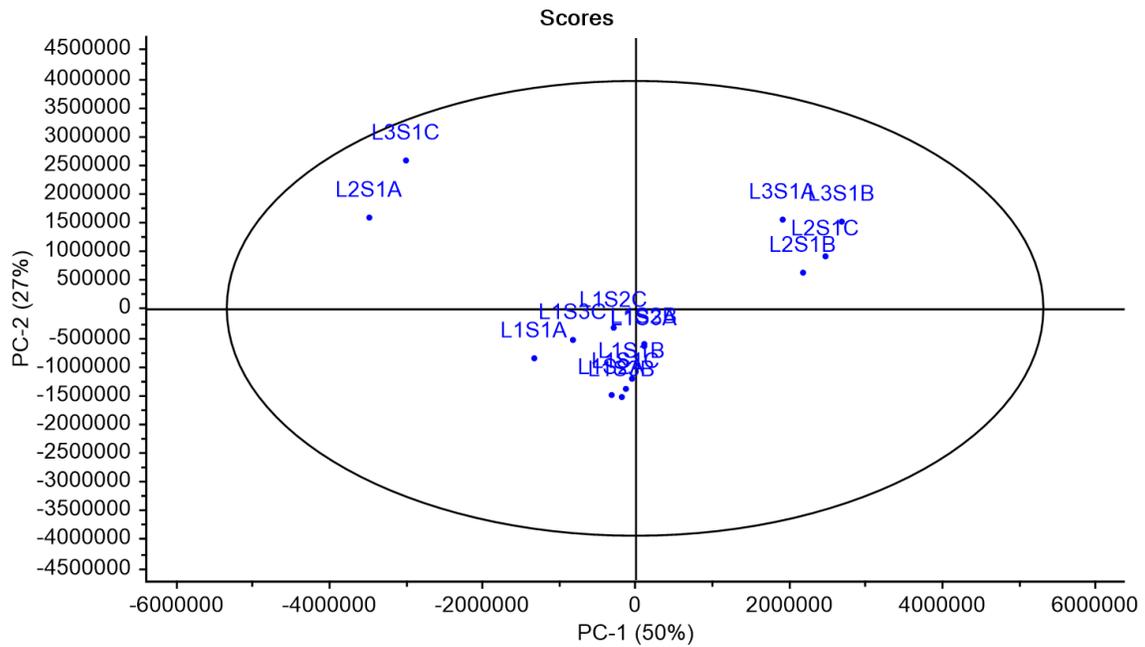


Figure 3. Dot-plot of the Principal component analysis. L = number of plant material supplier, S = number of batch.

Results show a distribution in three groups where the tinctures manufactured with the same plant material are located in the same group, but the change in plant material generates a different distribution within the point graph. The use of PCA analysis for the classification and quality control of herbal preparation is a wide application strategy, similar studies was been made with NMR spectra (Wang et al. 2004) and UHPLC-MS (Avula et al., 2014); however, NMR and mass spectrometry are more expensive techniques in relation with a HPLC-UV analysis.

The multivariate analysis of the chromatographic data confirmed the data obtained in the analysis of the physicochemical characteristics of the tincture where the similarity between the tinctures prepared with the same plant material is shown, whilst the similarities between the tinctures prepared with other plant materials is not shown. Moreover, a good correlation between the physicochemical characterization of the preparation and the chromatographic analysis combined with chemometric methods was shown. The latter being a faster, easier and more automatable strategy and therefore, more industrially applicable.

CONCLUSIONS

The tincture preparation method was demonstrated to be precise, however, it is not robust. The preparation characteristics are maintained as long as the plant material with which the tincture is made is not changed. This way, the preparation method is functional and traceable, but must be validated every time the source of the plant material is altered. The principal component multivariate analysis of chromatograms proved to be a useful, inexpensive and simple tool for the characterization and traceability of the *Matricaria recutita* tincture preparation method.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by a grant of Consejo Nacional de Rectores (CONARE, CNR-351-08) of Costa Rica.

<http://jppres.com/jppres>

REFERENCES

- Avula B, Wang YH, Wang M, Avonto C, Zhao J, Smillie T, Rua D, Khan I (2014) Quantitative determination of phenolic compounds by UHPLC-UV-MS and use of partial least-square discriminant analysis to differentiate chemo-types of Chamomile/*Chrysanthemum* flower heads. *J Pharm Biomed Anal* 88: 278-288.
- Barene I, Daberte I, Zvirgzdina L, Vilhelmine I (2003) The complex technology on products of German chamomile. *Medicina* 39(2): 127-131.
- British Pharmacopoeia (BP) (2008) London: British Pharmacopoeia Commission.
- Carmona R, López O, González M (2009) Relación entre índice de refracción y sólidos totales en extractos acuosos de *Calendula officinalis* L. (caléndula) y *Ocimum sanctum* L. (albahaca morada). *Rev Cubana Plant Med* 14(3): 23-28.
- Gan F, Ye R (2006) New approach on similarity analysis of chromatographic fingerprint of herbal medicine. *J Chromatogr A* 1104: 100-105.
- Gong F, Wang B, Liang Y, Chau F, Fung Y (2006) Variable selection for discriminating herbal medicines with chromatographic fingerprints. *Anal Chim Acta* 572: 265-271.
- Khan I, Smillier T (2012) Implementing a "Quality by Design" approach to assure the safety and integrity of botanical dietary supplements. *J Nat Prod* 75: 1665-1673.
- Lushear J (2005) New Orleans pharmacy museum: Preparing medicinal herbs. *Semin Integr Med* 3(4): 111-112.
- Padró L, López T (2000) Elaboración y caracterización de tinturas al 10, 20 y 30%, a partir de hojas y tallos de la especie *Pedilanthus tithymaloides* (L). *Poit. Rev Cubana Quim* 12(3): 30-36.
- Padró L, Marín J (2008a) Estandarización de los parámetros de calidad de la tintura de ítamo real 20%. *Rev Cubana Quim* 20(1): 19-23.
- Padró L, Marín J (2008b) Evaluación preliminar de la estabilidad física, físico-química y microbiología de la tintura de ítamo real 20%. *Rev Cubana Quim* 20(1): 24-30.
- Pribitkin ED (2005) Herbal medicine and surgery. *Semin Integr Med* 3(1): 17-23.
- Siries E, Nieto O, Sánchez N (2009) Evaluación de propiedades físico-químicas de tinturas de *Quassia amara* y *Maytenus ilicifolia*. *Rev Cubana Farm* 43(4): 85-92.
- Srivastava J, Shankar E, Gupta S (2010) Chamomile: An herbal medicine of the past with a bright future. *Mol Med Rep* 3: 895-901.
- The United States Pharmacopeial Convention (USP) (2014) United State Pharmacopeia 37 and National Formulary 32. Rockville: The United States Pharmacopeial Convention.
- Wang Y, Tang H, Nicholson J, Hylands P, Sampson J, Whitcombe I, Stewart C, Caiger S, Oru I, Holmes E (2004) Metabolomic strategy for the classification and quality control of phytomedicine: a case study of chamomile flower (*Matricaria recutita* L.). *Planta Med* 70(3): 250-255.
- World Health Organization (WHO) (2011). The World Medicines Situation 2011. Geneva: WHO Press.
- Xie B, Gong T, Tang M, Mi D, Zhang X, Liu J, Zhang Z (2008) An approach based on HPLC-fingerprint and

chemometrics to quality consistency evaluation of *Liuwei Dihuang* Pills produced by different manufacturers. J Pharm Biomed Anal 48: 1261-1266.
