Proceedings

Safe Processing, Optimization and Characterization of Natural Curcuminoids, Particularly Curcumin †

Toma Nardjes Mouas [1],* Nawel Ziani [2] and Saida Touzouirt [3]

[1] Université frères Mentouri-Constantine 1, Laboratoire d'Obtention de Substances Thérapeutiques LOST, Campus Chasbet Ersas, 25000 Constantine, Algeria

[2] Université Ferhat Abbes Sétif-1, Faculté des Sciences, Laboratoire de Chimie-Ingénierie des Matériaux et Nanostructures, Algeria; e-mail@e-mail.com

[3] Université Mouloud Mammeri, Tizi-Ouzou, Laboratoire des Ressources Naturelles, Algeria; e-mail@e-mail.com

* Correspondence: mouas.toma.nardjes@umc.edu.dz

† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://iecag2021.sciforum.net/.

Abstract: The introduction of new species in Algerian biodiversity is a crucial task in our environmental and economical politic, thus some spices of great interest as curcuma, saffron...are introduced by young start-ups as Algerian soil and climate are compatible with its culture; and in the framework of enhancement of such spices, the safe recovery of Curcumin in a keto-enol tautomeric form, a sensitive and versatile pro-oxidant and antioxidant propriety rarely reported, which improve its solubility and promote the effectiveness of Curcumin as natural anticancer agent, is reported. To attend our target, non-thermal and conventional solid-liquid extraction by cold maceration with several solvents' systems and time depending is used, furthermore an optimization using Response Surface Method (RSM) is conducted, then obtained crud is separated using LC chromatography using ethyl acetate/hexane gradient, pure compounds were identified by TLC, MP, UV, IR, and ^{1}H NMR spectroscopy. Experimental and optimized results reveled the best solvent and time: Acetone + shaking/6h, for extracting maximum yield of 7.52% and 7.6% respectively. Besides, obtained Curcuminoids were selectively separated from crud using as supplementary treatment oleoresins solubility in Ether Petroleum propriety, pure Curcumin in tautomeric form was identified, and an improved total yield of purified Curcumin (2.53%) is obtained. This eco-friendly, economic and easy process improved Curcuminoids and Curcumin recovery, preservation and efficiency for further industrial applications: building bloc in drug design as anticarcinogen agent, derma-cosmetics, functional foods, nutraceutics, hemysynthesis, and natural dyes which make great profits of introduction of this spicy in Algerian soil and Biodiversity.

Keywords: *Curcuma longa* L. specie; Curcumin; extraction kinetics; RSM; structure analysis

1. Introduction

Turmeric was widely used since long centuries in traditional medicine [1,2]. Nowadays, scientific researches proved that the main constituent of Curcuma species "Curcumin" is responsible of the biological activity of turmeric such as anti-inflammatory, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic, anti-cancer and anti-cardiovascular diseases [3–6].

These properties have been attracting for a long time the interest of various scientists, since this natural product with multi-targeted effects have less adverse effects than synthetic drugs with non observed toxicity even when taken at very high dose over long time [7,8], however these therapeutically effects are limited by a luck of solubility due to its diketo chemical structure, and one way of recent researches for improving its physico-chemical proprieties and reactivity, is structural modulation and transformation, another

way is to find an appropriate medium to generate a keto-enol tautomeric form and stabilize it.

Therefore, to meet industry demands for these biobased compounds with high quality, extraction is by far the most important stage in the recovery process, and depending on different factors such as high productivity, integrity and selectivity towards the target compound must be considered [9]. Extraction efficiency also depends on the localization and nature of the polyphenols. Conventional solvent extraction is the most widespread technique used at laboratory and at industrial scale to extract bioactive compounds from plant matrix, among which cold maceration presents the advantage of increasing mass transfer process with lower energy consumption and higher physical properties, in addition to sensible and volatile food compounds preservation.

In this context, the present study report an economic and Eco- friendly extraction of natural curcuminoids by cold maceration using: solvents' polarity, physical proprieties (shaking) and time depending, Insilco optimization of this method using RSM is also given. Furthermore, a selective extraction, isolation, purification and identification of main curcuminoids are investigated to prove the efficiency of the optimized matrix in the recovery of these prized bioactive compound in the target keto-enol form to reach and assess its optimal therapeutically effect and increase its exploitation possibility.

2. Experiments

2.1. Plant Materials

Turmeric rhizomes were commercially purchased from a local traditional shop, cleaned, dried and sprayed into a powder with an electrical pulvirisator until content was about 20 g dry weight. All other solvents and reagents used in this work were of analytical grade and purchased from Sigma-Aldrich Company Ltd. (Germany).

2.2. Extraction

Solvent extraction was carried out using cold maceration solid-liquid technique, where different organic solvents' polarity and extraction times were used to obtain optimum conditions.

Briefly, it consisted into the extraction of the hole crud of rhizomes (20 g) with 70 mL of Ethanol, Chloroform, Dichloromethane, Ethyl Acetate, Acetone and Acetone with shaking namely S1, S2, S3, S4, S5, S6 respectively, under magnetic agitation for 3, 4, 5, 6 h, then filtered with a Büchner leading to 24 crud extracts, the filtrates were evaporated to concentrates using rotary evaporation at 40 °C and stored in an amber coated bottle at 4 °C. The extraction yield was calculated as follows:

$$y = m_1/m2 \times 100$$

where y is the yield of dried extract, m_1 is the mass of dried extract; m_2 is the mass of the sample.

2.3. Optimization of Curcuminoids Extraction by Response Surfaces Method (RSM)

One of the objectives of this study is the optimization of the extraction yield of Curcuminoids using solid-liquid extraction by cold maceration in order to maximize the recovery of these bioactive compounds for industrial purpose. The optimal conditions are determined using central composite designs (CCD) [10]. Runs were performed by varying the extraction time and solvents' systems. The ratio of the mass/volume was considered constant during the experiments.

2.4. Central Composite Design

In this study, the central composite design consists of two-level factors. The extraction yield of curcuminoids represents the response Y that is closely related to the extrac-

tion time and solvents. Extraction time is proportional to the extraction yield; it is a quantitative variable and considered as an important factor because it directly affects the yield. The second qualitative variable is solvents' systems, chosen because to their influence on extraction selecting of bioactive compounds and increasing yield. The experiment matrix as well as the statistical analysis of the results was made using MODDE software, version 6 [11].

The number of trials N is taken from full factorial design (NF = 2k). Three additional points were added to the center of the domain (N0) in order to evaluate the reproducibility of the response and the experimental error and others points "Axial" for the quantitative factor.

$$N = NF + N\alpha + N0; N = 24 \text{ experiences}$$

The Equation (1) is given as follows

$$Y = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2 + e$$

Y represents the yield of crud curcuminoids;
X_1 and X_2 are the coded variables respectively for the extraction time and the solvents' system;
a_{ij} are the coefficients of the model (i = 0, 1, 2 and j = 1, 2).

3. Isolation of Curcuminoids

3.1. Ether Petroleum Treatment

A second step using Petroleum Ether came to extract more selectively curcuminoids from the best extract yield and separate them from oleoresins which are more soluble in Petroleum Ether. For this, obtained crud was mixed three times with 15 mL of Ether Petroleum. Following extraction, the remaining solvent was removed using rotary evaporation at 40 °C and stored in an amber coated bottle at 4 °C. The extraction yield was calculated as follows:

$$y = m_1/m2 \times 100$$

where y is the yield of dried extract, m_1 is the mass of dried extract, $m2$ is the mass of the Sample.

3.2. Thin Layer Chromatography

A rapid screening of suitable solvent system for isolating maximum compounds of turmeric rhizomes extract was done using thin layer chromatography (TLC). A chromatographic tank was filled with several solvents' systems to screen which is the best for curcuminoids resolution, and kept covered for 10 min to get saturated with solvent's vapors. Silica Gel 60 F254 precoated aluminum TLC plate 10 × 2 cm (Merck, Germany) was used as a stationary phase. The crude concentrated turmeric rhizomes extract was loaded on the TLC plate and put in the tank containing solvents. Physic revelation of spots was done using U.V lamp at 254 and 365 nm, Rf values were then calculated according to Touchstone [12].

3.3. Column Chromatography

Isolation of curcuminoids from Turmeric was done through column chromatography using the mobile phase selected on the basis of TLC screening. A glass column (100 × 3 cm) was filled with silica gel powder Kieselgel Merck (60–120 mesh), and was then loaded with hexane. Afterwards, concentrate crude extract was added on top with a pipette. The compounds of rhizomes extract were isolated using a gradient of 0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% ethyl acetate in hexane as mobile phase, with a drip elution rate. Afterward, these isolated fractions were analyzed through TLC again. The isolated

fractions containing the same compounds based on Rf values were pooled together, and fractions having a single spot on TLC plates were subjected to characterization and identification analysis.

3.4. Identification and Characterization

- Fourier transform infrared spectroscopy (FT-IR) analysis

    For Infrared (IR) spectra of isolated compounds, 1 mg of powder was mixed with KBr to form a plate. Absorption peaks of functional groups were recorded using Fourier transform spectrometry instrument SHIMADZU HYPER, FTIR-8201 PC in the range of 4000–400 cm^{-1}.

- UV-VIS spectroscopy

    UV-Visible absorbance was performed in scan mode with a SHIMADZU UV 16A spectrophotometer.

- Melting point

    The melting points of isolated compounds were recoded on a digital apparatus type STUART.

- Nuclear magnetic resonance spectroscopy (^{1}H-NMR)

    1D-1H-nuclear magnetic resonance (NMR) spectrum of the isolated and purified compounds was performed using NMR (Bruker Avance), 400 MHz, in CdCl$_3$, 1H chemicals shifts (δ) are given in ppm (the residual peak of deuterated solvent was used as reference).

3.5. Statistical Analysis

Each experiment was performed in five replicates and the data was subjected to calculations of mean ± S.E. The mean values were used for drawing the graphs.

4. Results and Discussion

4.1. Extraction Solvents Screening and Extraction Yields

According to obtained results we can observe that acetone with shaking (S6) exhibit the best extract yield from the first recorded hours (3 h) till the end (6 h)with 7.52% of yield extraction, this exhibit the role of shaking process in the efficiency of the recovery of target compounds, this could be explained by its facilitation of curcuminoids mass transfer from the matrix to the solvent, in fact conventional methods operate through cell permeation followed by solubilizing the active constituents by the extracting solvent. Curcumin present inside the oleoresin cells which in turn is covered by tightly packed cork cells probably makes the entry route for the solvent tougher [13], so pumice stone shaking process probably facilitates entry of the extracting solvent to solubilize out the target compound, thus lead to faster and efficient extraction.

Furthermore, the choice of Acetone solvent to get better extraction yield, is in line with previous studies that reports acetone as better solubilizing and extraction solvent than ethanol for Curcuminoids in conventional methods as well as in non-conventional methods [14], the two factors combination in one system S6 increased extract yield in shorter time to maximize the recovery of target compounds with more selectivity, efficiency and quality (stability).

4.2. Optimization by RSM

4.2.1. Statistical Study of Extraction Results

The obtained results by central composite design gave the best yield for acetone/shaking system, with 7.52% yield during 6 h of extraction.

4.2.2. Validation of the Mathematical Model

The validity of the mathematical model was done using MODDE 6 software.

4.2.3. Adequacy of the Model

The experimental values are nearly close to those predicted. The coefficients values of $R2$ and $Q2$ are 0.976 and 0.950, respectively. These values are close to equal, so the model is adequate.

4.2.4. Significant Factors

According to significance level of 5%, all solvents except S1 (ethanol) are significant, among which S3 (dichloromethane), S4 (ethyl acetate), and S5 (acetone) influence negatively, while S2 (chloroform) and S6 (acetone with shaking) influence positively the extract yield.

Consequently, only time, solvent S2 (chloroform) and S6 (acetone with shaking) are considered as significant factors for the extraction and positively influence extraction yields.

4.2.5. Analysis of Variance

The model is considered valid when the experimental Fisher F (Fobs) is greater than tab Fisher F (Fcrit); the p-value is close to zero. Therefore, the model is statistically significant and should be suitable for given response.

4.2.6. Residues Study

Evolution of residues with the studied factors, shows random dispersion of residues.

Thus, it indicates that the (acetone/shaking) system extraction during 6hours is the most suitable to get the best possible extraction yield.

4.3. Optimization

The optimum yield was obtained for (acetone/shaking) with 7.6% yield during 6 h of extraction.

5. Isolation of Curcumin from Turmeric Rhizome Extract

Rhizomes' extract (395 mg/1.98% yield) obtained after selective extraction with S6 and ether petroleum treatment, was subjected to isolation of bioactive compounds through TLC and column chromatography. Hexane: ethyl acetate (70:30; *v:v*) solvent system gave a good resolution for isolating curcuminoids on TLC plate. The gradient solvent system used in column chromatography isolation lead to elute a total of 9 fractions of 50 mL each. Only F3, F4, F5 gave a single, yellow colored spot, whereas the other fractions gave multiple spots, F4 with a R*f* value of 0.78 corresponding to the main standard compound Curcumin, was obtained with 2.53% of yield better than the conventional Soxhlet assisted acetone after 8 h of extraction resulted in a 2.1% Curcumin yield [14], is consequently chosen for further characterization and validation using 1H-NMR, FT-IR, melting point and UV-VIS analysis.

Identification and Characterization of Purified Curcumin through 1HNMR, FT-IR, Melting Point and UV-VIS

The 1H-NMR spectrum of the isolated compound F4 exhibit six peaks regions: C, H, D, E, F, G [15–17]. Characteristic of Curcumin functions, Therefore, the isolated compound F4 was identified as [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-hepta diene-3,5-dione].

Although, Curcumin is commonly named as 1,7-bis (4-hydroxy-3-méthoxyphényl)-1,6-hépatadiène3,5-dione, highlighting its dikitone tautomeric form, however, few studies

reported also two other possible rare forms asymmetric kito-enol tautomers attributed to its interactions with acid ph medium and solvents like CDCl3 [17,18].

Indeed, apparition of a doublet in region I (6.4–6.5 ppm) indicating the presence of non equivalent proton and the appearance of a $H_{hydroxyl}$ at 4.1 ppm suggests the kito-enol forms of Curcumin.

The importance of the tautomerism of Curcumin has been examined in a study investigating the molecular mechanism of the observed synergy between Curcumin and water soluble antioxidants in cancer chemoprevention at physical conditions [19]. It has been observed that the Curcumin radical preferentially exists as a phenoxyl-type species, which is more hydrophilic than the keto form. Being more hydrophilic, it is preferentially moved to the external side of the cell membrane; this effect is probably responsible of Curcumin effectiveness as a scavenger of carcinogenic free radicals [20].

- The FT-IR spectra of isolated F4 corresponding to the main standard Curcumin revealed characteristic absorption peaks in KBr pallets at 1510 cm^{-1} (C=O), 3500 cm^{-1} (OH aromatics function), 1596 cm^{-1} (C=C aromatics), 1029 cm^{-1} (C-O-C) et 1278 (C-O enol) [21,22].
- Melting point

F4 Melting point was observed at 183.5 °C, which indicate the purity of obtained Curcumin.

- *UV-VIS*

UV-VIS spectra of isolated Curcumin (F4) was performed in ethyl acetate solution and recorded in the region of 800–200 nm at room temperature, it reveled two absorption bands around 417nm and 263 nm, corresponding to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively, while it shows one absorption at 429 nm in methanol, which is in nice agreement with [23].
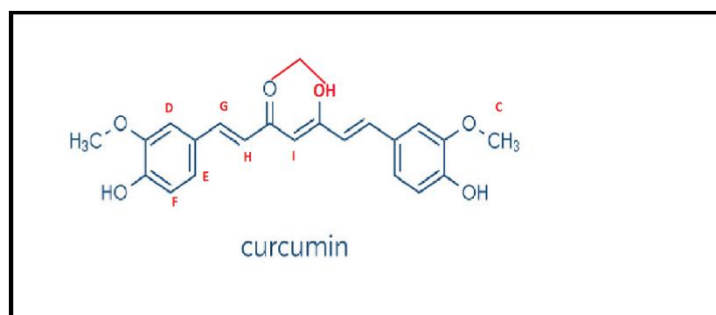
Figure 1. Tautomeric form of Curcumin.

6. Conclusions

Conventional methods still interesting extracting methods prized at industrial scale, because it is known to be safe, economic, non-thermal and easy extraction method; besides, when optimized using several solvent systems and time in addition to a supplementary treatment, it could be very interesting method to maximize the recovery of thermofragile natural biomolecules particularly Curcumin from turmeric rhizomes and its structure stability preservation as building bloc for industrial purpose. The present study highlighted the role of solvent medium in the selectivity and structural stability of curcuminoids using polarity proprieties in extraction, mechanic propriety of shaking and time as decisive factors in improving the recovery of target bioactive compounds. In fact, shaking extractions under optimized process control using experimental design methodology through central composite design exhibit nearly the same result for predicted and experimental yields namely acetone with pumice stone during 6 h to have 7.6% crud which is more than most reported results in literature. Furthermore, this selective extraction and treatment lead also to maximize pure Curcumin recovery (2.52%), isolated Curcumin was identified

through TLC, melting point, IR, UV and NMR 1H spectroscopy which reveled the target keto-enol tautomery, a very fragile and rare form, giving a versatile propriety to Curcumin reactivity as pro-oxidant and antioxidant at the same time; this effect is probably responsible of Curcumin effectiveness as a scavenger of carcinogenic free radicals, promoting it as anticancer agent for further applications.

Author Contributions: T.N.M. conceived and designed the experiments, analyzed the data and wrote the paper; N.Z. and S.T. performed the experiments; analyzed the data. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Gupta, S.C.; Kismali, G.; Aggarwal, B.B. Curcumin, a component of turmeric: From farm to pharmacy. *BioFactors* **2013**, *39*, 2–13, doi:10.1002/biof.1079.
2. Ouelbani, R.; Bensari, S.; Mouas, T.N.; Khelifi, D. Ethnobotanical investigations on plants used in folk medicine in the regions of Constantine and Mila (North-East of Algeria). *J. Ethnopharmacol.* **2016**, *194*, 196–218, doi:10.1016/j.jep.2016.08.016.
3. Ahsan, H.; Parveen, N.; Khan, N.U.; Hadi, S. Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. *Chem. Interact.* **1999**, *121*, 161–175, doi:10.1016/s0009-2797(99)00096-4.
4. Péret-Almeida, L.; Cherubino, A.; Alves, R.; Dufossé, L.; Glória, M. Separation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Res. Int.* **2005**, *38*, 1039–1044, doi:10.1016/j.foodres.2005.02.021.
5. Cikrikci, S.; Mozioglu, E.; Yilmaz, H. Biological activity of curcuminoids isolated from Curcuma longa. *Rec. Nat. Prod.* **2018**, *2*, 19–24.
6. Campbell, M.S.; Fleenor, B.S. The emerging role of curcumin for improving vascular dysfunction: A review. *Crit. Rev. Food Sci. Nutr.* **2017**, *58*, 2790–2799, doi:10.1080/10408398.2017.1341865.
7. Li, H.; Sureda, A.; Devkota, H.P.; Pittalà, V.; Barreca, D.; Silva, A.S.; Tewari, D.; Xu, S.; Nabavi, S.M. Curcumin, the golden spice in treating cardiovascular diseases. *Biotechnol. Adv.* **2020**, *38*, 107343, doi:10.1016/j.biotechadv.2019.01.010.
8. Lao, C.D.; RuffinIV, M.T.; Normolle, D.; Heath, D.D.; Murray, S.I.; Bailey, J.M.; Boggs, M.E.; Crowell, J.; Rock, C.L.; Brenner, D.E. Dose escalation of a curcuminoid formulation. *BMC Complement Altern. Med.* **2006**, *6*, 10, doi:10.1186/1472-6882-6-10.
9. Osorio-Tobón, J.F.; Carvalho, P.I.; Rostagno, M.A.; Petenate, A.J.; Meireles, M.A.A. Extraction of curcuminoids from deflavored turmeric (Curcuma longa L.) using pressurized liquids: Process integration and economic evaluation. *J. Supercrit. Fluids* **2014**, *95*, 167–174, doi:10.1016/j.supflu.2014.08.012.
10. Goupy, J.; Creighton, L. *Introduction aux Plans D'expériences*, 3 ed.; DUNOD: Paris, France, 2006; p. 336.
11. Software, M. 2001, Umetrics AB.
12. Touchstone, J.C. *Practice of Thin Layer Chromatography*, 3rd ed.; John Wiley & Sons: Hoboken, NJ, USA, 1992; ISBN 0471612227, 9780471612223, 400 p.
13. Mandal, V.; Mohan, Y.; Hemalatha, S. Microwave assisted extraction of curcumin by sample–solvent dual heating mechanism using Taguchi L9 orthogonal design. *J. Pharm. Biomed. Anal.* **2008**, *46*, 322–327, doi:10.1016/j.jpba.2007.10.020.
14. Wakte, P.; Sachin, B.; Patil, A.; Mohato, D.; Band, T.; Shinde, D. Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. *Sep. Purif. Technol.* **2011**, *79*, 50–55, doi:10.1016/j.seppur.2011.03.010.
15. Nagahama, K.; Utsumi, T.; Kumano, T.; Maekawa, S.; Oyama, N.; Kawakami, J. Discovery of a new function of curcumin which enhances its anticancer therapeutic potency. *Sci. Rep.* **2016**, *6*, 30962, doi:10.1038/srep30962.
16. Sarika, P.; Nirmala, R.J. Curcumin loaded gum arabic aldehyde-gelatin nanogels for breast cancer therapy. *Mater. Sci. Eng. C* **2016**, *65*, 331–337, doi:10.1016/j.msec.2016.04.044.
17. Payton, F.; Sandusky, P.; Alworth, W.L. NMR Study of the Solution Structure of Curcumin. *J. Nat. Prod.* **2007**, *70*, 143–146, doi:10.1021/np060263s.

18. Roughley, P.J.; Whiting, D.A. Experiments in the biosynthesis of curcumin. *J. Chem. Soc. Perkin Trans.* **1973**, *1*, 2379–2388, doi:10.1039/p19730002379.

19. Jovanovic, S.V.; Boone, C.W.; Steenken, S.; Trinoga, M.; Kaskey, R.B. How Curcumin Works Preferentially with Water Soluble Antioxidants. *J. Am. Chem. Soc.* **2001**, *123*, 3064–3068, doi:10.1021/ja003823x.

20. Euterpio, M.A.; Cavaliere, C.; Capriotti, A.L.; Crescenzi, C. Extending the applicability of pressurized hot water extraction to compounds exhibiting limited water solubility by pH control: Curcumin from the turmeric rhizome. *Anal. Bioanal. Chem.* **2011**, *401*, 2977–2985, doi:10.1007/s00216-011-5383-7.

21. Mohan, P.K.; Sreelakshmi, G.; Muraleedharan, C.; Joseph, R. Water soluble complexes of curcumin with cyclodextrins: Characterization by FT-Raman spectroscopy. *Vib. Spectrosc.* **2012**, *62*, 77–84, doi:10.1016/j.vibspec.2012.05.002.

22. González-Albadalejo, J.; Sanz, D.; Claramunt, R.M.; Lavandera, J.L.; Alkorta, I.; Elguero, J. Curcumin and curcuminoids: Chemistry, structural studies and biologicalproperties. *An. Real Acad. Farm.* **2015**, *81*, 278–310.

23. Goel, A.; Kunnumakkara, A.B.; Aggarwal, B.B. Curcumin as "Curecumin": From kitchen to clinic. *Biochem. Pharmacol.* **2008**, *75*, 787–809, doi:10.1016/j.bcp.2007.08.016.