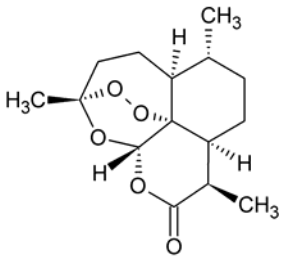
Comparative Assessment of Technologies for Extraction of Artemisinin

A summary of report commissioned through
Malaria Medicines Ventures (MMV)



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Introduction

Traditionally the majority of *Artemisia annua* grown worldwide is processed through solvent extraction, using hexane and petroleum ether. The only other considered alternative has been super critical CO_2. Whilst petroleum ether and hexane are cheap to buy, both solvents represent a considerable safety hazard and could be harmful to the environment.

Following the world wide exposure *Artemisia a.* has received as a treatment for malaria, a number of other technologies which claim to have greater efficiency, are safer or more environmentally friendly, are now being promoted. These processes were initially developed for the extraction of essential oils, fragrances and other pharmaceutical products, but initial trials on *Artemisia a.* have been successful and indicate that they could be used as an alternative to the existing technologies.

The lack of accurate data on hexane extraction, together with the emergence of these new technologies, makes it difficult for new and existing Artemisia producers to assess the efficiency, financial viability, safety and environmental impacts of the individual processes, and thereby select the process which is best for their application.

The main objectives of this study have therefore been to highlight and examine the new technologies which could be used for the extraction of artemisinin and to develop a benchmarking procedure through which these new technologies can be compared to existing extraction methods e.g. hexane.

The following review is based on the full paper which has been submitted for peer review and publication in the Journal of Natural Products. A link to the full paper will be included on this website as soon as it has been published.

Executive Summary

Extraction of artemisinin from *Artemisia annua* is currently mainly performed using hydrocarbon extraction processes. Due to the risk to human health, poor environmental performance, and the dangers of processes involving large volumes of volatile combustible fluids, there is a need to develop alternative processes that would be able to compete in terms of efficiency and cost, and have little or none of the drawbacks associated with hydrocarbon solvents. Extraction with supercritical carbon dioxide ($scCO_2$), ethanol, ionic liquids, and hydrofluorocarbon HFC-134a are compared against the baseline case of extraction with hexane-ethyl acetate mixed solvent. For a variety of reasons a limited number of other solvents and/or technology providers have not been included, as insufficient data was forthcoming,

The study identified a number of key parameters relevant to the majority of stakeholders. These parameters were used in the multi objective assessment of the extraction technologies. Main parameters included efficiency of primary extraction, potential for developing a compact 'back-of-a-truck' plant, capital cost of plants with specific biomass throughput, risk to human health and the environment, and potential for multi-crop operation. Amongst the compared alternative technologies, HFC-134 based extraction has been commercialised on the pilot (0.5 m^3 vessels) and low-throughput industrial (1 m^3 vessels) scales, $scCO_2$ extraction has been demonstrated on 1 L scale and pilot tests are underway. Considerable discrepancy in the available data on ethanol extraction was identified. Extraction of natural products with ionic liquids is an emerging technology and only initial lab tests data was available. Therefore, the study compares the performance of a developed optimised process (hexane extraction) with performance of developing processes ($scCO_2$ and HFC-134a) with that of emerging new technology (ionic liquids).

Extraction with HFC-134a and ionic liquids were shown to be the most promising replacement for hexane extraction. Ionic liquids have the potential to outperform hexane extraction in all main criteria. However, further research is urgently needed to develop methods of efficient regeneration of the solvent, its recovery from the spent biomass and optimisation of the ratio of solvent to biomass. Extraction with HFC-134a provides much cleaner extracts with higher concentration of artemisinin and therefore should lead to simpler recovery of artemisinin from the primary extract. The process is economic, low risk and has low environmental impact, with the main concern being the need for tight control of the solvent inventory and of its recovery from the spent biomass. Extraction with ethanol was shown to be consistently worse than that with hexane.

The main area of uncertainty remains the process of recovery of artemisinin from the primary extracts produced by the different extraction processes.

General aspects of artemisinin extraction

Artemisinin compounds have been predominately found in the upper parts of the *Artemisia annua* plant, with the concentration of artemisinin said to peak just before or during full flowering, the difference being attributed to climatic conditions, plant variety, or other, yet undetermined, factors.[1] More specifically, artemisinin and its precursor artemisinic acid have been shown to be localised in the glandular trichomes on the leaf surface.[2,3] The main consequences of this are that (*i*) it may not be necessary to mechanically crush the plants prior to extraction for reasons other than to increase the packing density, and (*ii*) the artemisinin content depends on the age of the leaf, since in older leaves the glands were often found to be ruptured. Repeated harvesting of young leaves from the same plant was shown to considerably increase the amount of artemisinin produced per area.[4] Due to the physico-chemical properties of artemisinin (low thermal and chemical stability of the endoperoxide function, low polarity and, hence, poor solubility in water and good solubility in organic solvents – see Annex III, Table 1), its extraction with non-polar solvents is necessarily complicated by simultaneous extraction of essential oils, chlorophylls and waxes. Therefore, the extraction step must be followed by separation of artemisinin from the initial liquor. This is generally achieved by sequential crystallisation from an alcohol solution. Figure 1 shows the processing steps for artemisinin extraction by hexane-ethyl acetate mixed solvent with an identified system boundary for comparative assessment. The step of separation of artemisinin from the raw extract is common to all extraction processes, however, there are significant variations in the times and purification steps. See Annex III, Table 2, for artemisinin properties according to the monograph and Table 3, typical buyer specification..

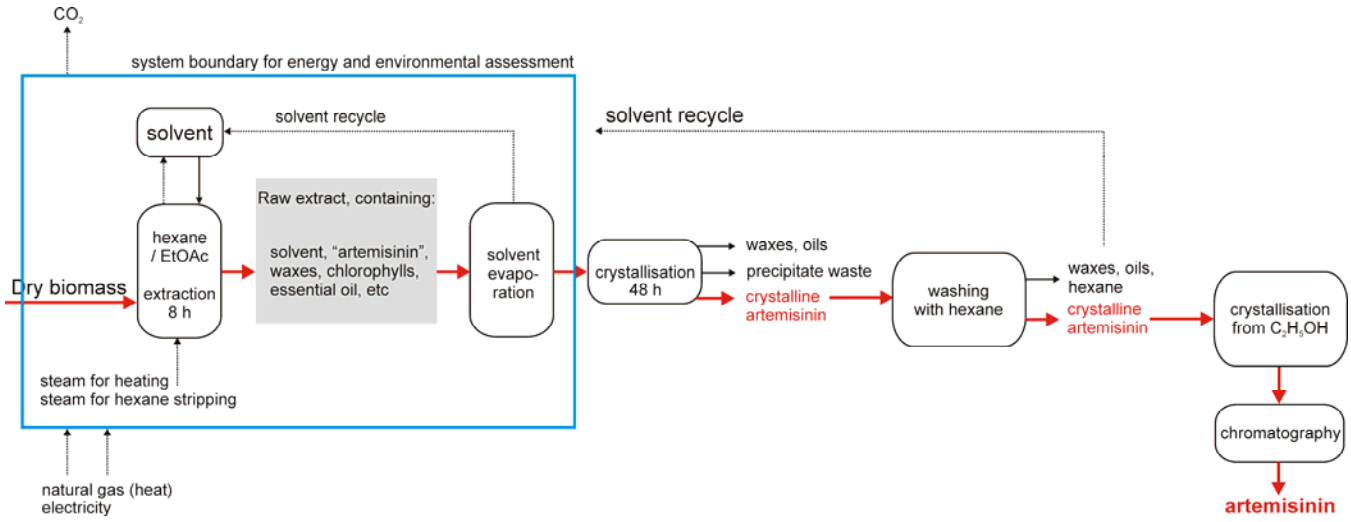

Figure 1. Scheme of artemisinin extraction with hexane-ethyl acetate mixed solvent.

Baseline process: extraction with hexane/petroleum ether

In the simplest **hexane** batch extraction, dried crushed leaf is soaked three-four times in fresh portions of warm (30 – 45 ºC) hexane or petroleum ether, each extraction cycle taking between 10-48 hours.[5,6] Under flow conditions (solvent percolation through packed biomass bed) at the same temperature the duration of each cycle can be reduced to 90-120 min.[7] Different extraction regimes *i.e.*, batch, percolation and continuous are shown in Figure 2. In order to improve the efficiency of extraction, a small amount of co-solvent ethyl acetate can be added to the main non-polar hydrocarbon solvent. This increases the solubility of artemisinin in the solvent mixture by about two orders of magnitude.[8]

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[1] Laughlin, J. C.; Heazlewood, G. N.; Beattie, B. M. In *Artemisia*, Wright, C. W., Ed. Taylor & Francis: London and New York, 2002.
[2] Duke, M. V.; Paul, R. N.; Elsohly, H. N.; Sturtz, G.; Duke, S. O. *Int. J. Plant Sci.,* **1994**, *155*, 365-372.
[3] Duke, S. O.; Paul, R. N. *Int. J. Plant Sci.,* **1993**, *154*, 107-118.
[4] Kumar, S. *Natl. Acad. Sci. Lett.,* **2005**, *28*, 325-338.
[5] El-Sohly, H. N.; Jr, E. M. C.; El-Feraly, F. S.; El-Sherei, M. M. *J. Nat. Products,* **1990**, *53*, 1560-1564.
[6] Haynes, R. K. *Current Topics In Medicinal Chemistry* **2006**, 6, 509-537.
[7] Khanuja, S. P. S., Personal Communication, 2006.
[8] Reitz, H.; Hill, C. *Potential for the extraction and sale of artemisinin: Tanzania and/or Kenya*; TechnoServ Tanzania: Arusha, Tanzania, 5.10.2004, 2004.

Following extraction, the solvent is drained and spent biomass must be stripped of the residual solvent. Biomass is said to absorb solvent in the ratio of 1 $L \cdot kg^{-1}$.[9] Stripping of the solvent can be achieved by simple evaporation in air under natural convection, which is potentially hazardous and leads to the release of significant quantities of environmentally harmful volatile hydrocarbon, or more efficiently by steam stripping followed by condensation and recovery of solvent. The recovery and re-use of the solvent reduces the environmental impact and improves the cost-effectiveness of the process. Vacuum stripping may also be used to avoid potential biomass decomposition under steam and to avoid downstream water-solvent separation.

The obtained crude extract is flash-evaporated to 10 % of its initial volume and the remaining liquor is left to stand at ambient temperature over *ca*. 48 h to crystallise crude artemisinin, allowing decanting of the liquor. Crude artemisinin is washed with warm hexane to remove the waxes and other precipitated impurities. In order to remove the waxes artemisinin is re-crystallised several times from ethanol-water azeotrope (95 wt% ethanol) in the presence of activated carbon adsorbent, followed by vacuum evaporation.[9] Further purification is achieved by chromatography. An alternative method of separating artemisinin from the initial hexane extraction involves liquid-liquid extraction of artemisinin related compounds from hexane into acetonitrile.[5,10] This method is not considered due to the hazardous nature of acetonitrile to the environment and human health, rendering its large scale use unacceptable.

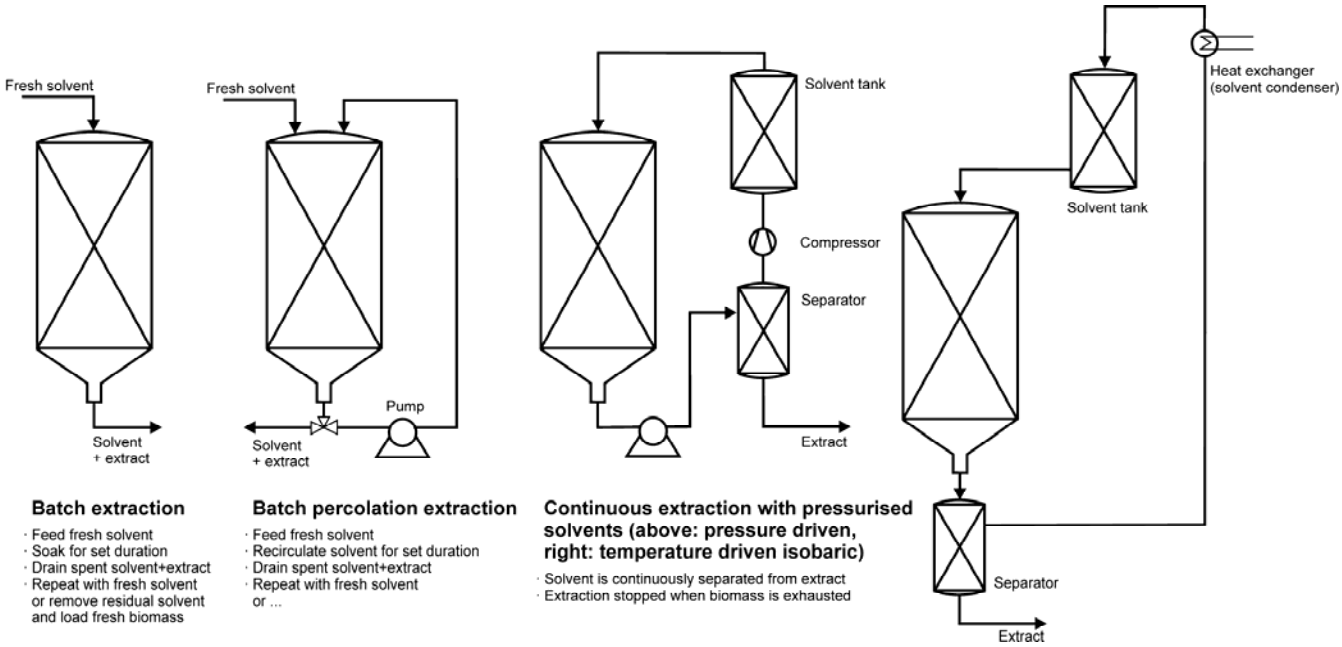


Figure 2. Schematic diagrams of extraction plant options.

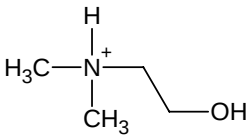
Batch and percolation extraction with alternative solvents: ethanol and ionic liquids

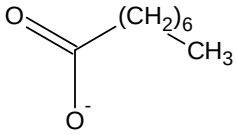
Organic **ionic liquids** (organic equivalent of molten salts) is a new class of solvents, characterised by negligible vapour pressure, nonflammability and possibility to tune solvation properties over a very broad range. Since ILs lack two major drawbacks of the hydrocarbon solvents: vapour pressure and flammability, these solvents are often cited as a 'green alternative'. Ionic liquids have been reported as a very good reaction medium for many organic reactions catalysed by chemical catalysts, as well as bio-catalysts. Despite a fairly recent development of this field of research, several chemical processes based on ionic liquids have already been commercialized. However, there are very few publications on the application of ionic liquids in extraction of bio-molecules, see review.[11] The assessment is based on the preliminary study undertaken by Bioniqs Ltd (UK) and funded by MMV.

Two out of five screened ionic liquids showed promising performance: N,N-dimethylethanolammonium octanoate (DMEA oct, **1**) and bis(2-methoxyethyl)ammonium bis(trifluoromethylsulfonyl)imide (BMOEA bst, **2**). The extraction process is similar to a standard liquid-solid extraction and was performed in a

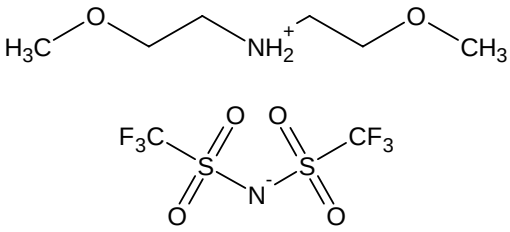
[9] Vries, D. P. J. d.; Chan, P. N. G., *Development and application of anti-malaria drugs, based on artemisinin, in Vietnam*. 1998.
[10] El-Feraly, F. S.; El-Sohly, H. N. 4,952,603, 28.08.1990, 1990.
[11] Zhao, H.; Xia, S.; Ma, P. *J. Chem. Techhl. Biotechnl.,* **2005**, *80*, 1089-1096.

batch regime at 25 ºC. Extraction with the DMEA oct solvent reached maximum solute concentration after 30 minutes of extraction. The observed concentration of artemisinin in solution was similar to that obtained in the benchmark experiment with hexane at the same temperature.





1 N,N-dimethylethanolammonium octanoate



2 bis(2-methoxyethyl)ammonium bis(trifluoromethylsulfonyl)imide

In the case of the solvent **2** the rate of extraction was considerably slower than that with **1**. However, the maximum concentration of artemisinin in solution was higher by 23 %.The obtained rate of extraction with **2** was similar to the rate of extraction with n-hexane at the same temperature. Thus, in comparison with hexane, ionic liquid **1** gave a similar efficiency of extraction at a considerably faster rate, whereas ionic liquid **2** gave a higher extraction efficiency at the same rate.

The process of separation of artemisinin from the raw extract involved partitioning with water at ambient temperature. This causes simultaneous separation of the oil fraction and crystallisation of artemisinin. Crystallisation allows a separation of 82 % of the total extracted amount of artemisinin; the remainder is assumed to be lost with the oil phase. The crystals are 95 % artemisinin (by NMR) and are essentially free of solvent (not detectable by NMR). Separation was achieved in about 10 minutes. Work is now being undertaken to further refine the process since there are alternative options for recovery of artemisinin from the primary extract and regeneration of ionic liquid solvent and solvent recovery from spent biomass had still to be optimised.

A recent study of extraction by **ethanol** aqueous azeotrope at room temperature claimed high efficiency of extraction.[12,] Ethanol is potentially an attractive solvent due to its wide spread availability from renewable feedstocks. This is especially important for processes that are predominantly focusing on locally sourced materials to improve the overall process sustainability. A potential constraint on the use of ethanol as a process solvent is its use as a spirit. This can be resolved by using a spiked solvent, which is the customary practice in the EU. However, there are similar concerns with the use of ethanol as in the case of hexane: it is a flammable solvent, with high toxicity and high risk in use.

The process based on ethanol extraction involves three sequential extractions with fresh solvent portions followed by flash evaporation of solvent to reduce the volume of the primary extract. Some process optimisation is possible to reduce the ratio of solvent to biomass, as described in the original publication. It should be stressed that a very recent study of ethanol extraction of artemisinin in a pressurised percolator extractor was unable to replicate high extraction efficiencies.[13]

Continuous extraction with supercritical CO_2 and hydrofluorocarbon HFC-134a

Extraction of artemisinin by **scCO$_2$** or **sub-critical liquid CO$_2$** has been described in the literature[14] and large-scale trials are currently being undertaken. The efficiency of extraction of artemisinin from biomass is reported to be quantitative, rapid and with higher selectivity compared with the hydrocarbon solvents extraction, based on the gram scale laboratory tests. However, there is wide variability in the efficiencies of extraction with $scCO_2$ cited in the open literature, dependent on the scale of extraction, use of co-solvents, temperature and pressure of extraction, and superficial velocity of the solvent in the extractor. Thus, a lower efficiency than that obtained with a hydrocarbon solvent was reported in the absence of a hydrophilic co-solvent,[14c] whereas an earlier patent[14b] gives a wide range of extraction efficiencies, between 25-100 %, depending on the operating conditions. Such a wide variation can be attributed to the accuracy of the analytical methods of determining artemisinin concentrations, variability in the operating conditions (pressure and temperature, duration of

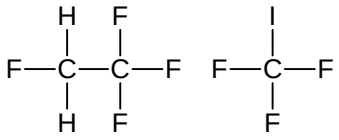
[12] Rodrigues, R. A. F.; Foglio, M. A.; Júnior, S. B.; Santos, A. d. S.; Rehder, V. L. G. *Quim. Nova* **2006**, *29*, 368-372.
[13] Freyhold, M. van, Personal Communication, 2006.
[14] (a) Kohler, M.; Haerdi, W.; Christen, P.; Veuthey, J.-L. *J. Chromatography A.,* **1997**, *785*, 353-360; (b) Wheatley, G. W.; Chapman, T. B. US 6,180,105B1, 2001; (c) Quispe-Condori, S.; Sánchez, D.; Foglio, M. A.; Rosa, P. T. V.; Zetzl, C.; Brunner, G.; Meireles, M. A. A. *J. Supercritical Fluids,* **2005**, *36*, 40-48; (d) Pulz, O. DE 10336056A1, 2005; Mengal, P.; Zwegers, J.; Monpon, B. French Patent 2,706,166, 1993.

extraction, concentration of co-solvent), variability in the water content of dry biomass, and variability between and within biomass samples. A typical value of overall extraction efficiency, including the secondary purification by crystallisation, is about 80 %. The duration of extraction cycle depends greatly on the scale of extraction, use of co-solvents as well as more specific aspects of extractor design that influence optimal solvent mass flow rate. Thus, 20 min extraction cycle was quoted for *ca*. 1 L scale in the case of $scCO_2$-ethanol system, whereas detailed kinetic study of artemisinin extraction with $scCO_2$ without co-solvents showed extraction times up to 2 h on a 0.2 L scale. A typical $scCO_2$ process scheme is shown in Fig 2 – continuous extraction, pressure driven.

Hydrofluorocarbon HFC-134a (1,1,1,2-tetrafluoroethane) is classed as non-flammable and has zero ozone depleting potential. It is amongst the most studied and utilised materials for which there is a life-cycle impact study.[15] In Europe, Japan and USA, HFC-134a is accepted by regulatory bodies for use as a solvent in the extraction of food flavourings. One drawback of the solvent is its high global warming potency factor – 1300 times larger than that of carbon dioxide. Therefore, complete recycle and capture of the solvent within a process is of significant importance.

Pilot plant for continuous extraction using HFC-134a at Ineos Fluor Ltd.

Hydrofluorocarbons are gases under normal conditions and are liquefied at relatively low pressures. Therefore, these solvents are ideally suited for continuous extraction processes, when de-pressurisation of the solvent results in a rapid separation of the extracted material. Because of the modest pressures and low operating temperatures, the energy required for continuous de-pressurisation/pressurisation cycle is not high, resulting in low energy costs, low operating costs, and low greenhouse gas emission due to energy duty. Furthermore, re-circulation of solvent can be achieved without pumps, by establishing an isobaric condensation-evaporation cycle, thus avoiding the need for expensive capital investment in the pump and the compressor. In this case the flow-rate of solvent depends on the efficiencies of condenser and evaporator, as well as percolation properties of the packed biomass, see process scheme in Figure 2. A commercial extraction plant (Phurua Natural Oils Limited) based on this principle has been operating in Thailand since 2004.[16]



3 **4**

Extraction of natural compounds by **(3)** 1,1,1,2-tetrafluoroethane (**HFC-134a**) and **(4)** iodotrifluoromethane (**ITFM**) have been reported in the literature,[17] although not specifically of artemisinin. Physical properties of ITFM and HFC-134a are quite similar. However, because of the presence of weaker C-halogen bond with iodine, there are potential toxicity issues with the use of ITFM. More specifically, acute toxicity of ITFM itself was found only in exposure to very high concentrations (>25 %vol), but there is a significant risk of cardiac sensitization at levels of exposure of 0.2 %vol (2000 ppm) and there are suspicions of potential carcinogenic effect.[18] ITFM has poor stability on sunlight, in the presence of artificial UV light and at temperatures above 100 ºC; its decomposition is facilitated in the presence of copper and moisture.[18b] The products of

A compact continuous extraction facility using HFC-134a. (Peter Wilde Associates Ltd.)

[15] McCulloch, A.; Lindley, A. A. *Int. J. Refrigeration* **2003**, *26*, 865-872.
[16] Wilde, P. F., Personal Communication, 2006.
[17] (a) Wilde, P. F. US Patent 5,512,285, 1996; (b) Wilde, P. F.; Skinner, R. E.; Ablett, R. F. WO 03/090520 A2, 2002; (c) Corr, S. *J. Fluorine Chem.,* **2002**, *118*, 55-67.
[18] (a) Iodotrifluoromethane: toxicity review. http://www.nap.edu/catalog/11090.html (09.07.2006); (b) McCain, W. C.; Macko, J. Toxicity review for iodotrifluoromethane (CF_3I). http://www.bfrl.nist.gov/866/HOTWC/HOTWC2003/pubs/R9902725.pdf (09.07.2006).

ITFM decomposition are HF, HI and COF_2 which are highly toxic themselves and can react further with organic matter leading to acute toxicity. Relatively poor stability of ITFM requires specific safety measures during storage and use. HFC-134a is a considerably more stable compound and has been subject to long-term (5 yrs) stability trials for its pharmaceutical applications.

Commercial HFC-134a based extraction facility, Phurua Natural Oils Ltd, Thailand. (Courtesy of Peter Wilde Associates Ltd).

There are also significant differences in the extraction efficiency and prices. For comparison, 100 g of HFC-134a and ITFM were quoted at £71.8 and £236 respectively by Aldrich catalogue in July 2006 (note that Aldrich prices cannot be used for scaling and only given for comparison purposes). Based on the data reported in the patent literature, ITFM is a much stronger solvent than HFC-134a - HFC-134a extracts little waxes and heavier oils, which are effectively extracted with the ITFM solvent. Due to this difference in solvation properties, HFC-134a is expected to be more selective towards artemisinin than ITFM. By combining ITFM with co-solvents, including HFC-134a, it is possible to regulate the extraction efficiency.

The data reported in this study are based on the information kindly provided by Ineos Fluor Ltd. These data are supported by similar results obtained by Peter Wilde Associates Ltd.

Comparison of extraction efficiency

Based on the available data for each extraction process, the efficiency of extraction relative to biomass artemisinin content was estimated. The other important criteria are the duration of the complete extraction cycle, running and capital cost. These data are shown in Table 1. Among these processes HFC-134a and ionic liquids based extractions may offer the cheapest running cost and competitive capital cost with the hexane extraction. Note, that capital cost includes the price of solvent inventory.

Table 1. Efficiency of extraction (mass artemisinin in crude crystalline extract relative to mass of artemisinin in biomass), duration of extraction cycle (extraction to biomass exhaustion + loading of fresh biomass), running cost (includes only the cost of electricity and natural gas for heating for the main extraction process), capital cost (includes only equipment for the main extraction and solvent inventory).

	Extraction efficiency / %	**Duration of extraction cycle / h**	**Running cost / €·kg^{-1} artemisinin**	**Capital cost for 2.5·10^6 kg (biomass)·annum^{-1} / m€**
Hexane	60	8-10	28	0.7
Ethanol	73	7	47	1.0
Ionic liquids	79	2.5-6	22	0.3-1.0
$scCO_2$	82	3-6	42	4.1
HFC-134a	>62	6	19	1.0

In order to compare the different extraction technologies, it is also necessary to compare the risk and safety, environmental performance (green house gas emissions), and potential risk to human health. These parameters were normalised against the hexane baseline case and shown in Figure 3.

Extraction with HFC-134a and ionic liquids compare best against hexane extraction. Green house gas emissions in the case of HFC-134a is due to the residual solvent in spent biomass, which represents annual loss of < 5 % of solvent inventory.

Based on the extraction cycle time, the feasibility of a mobile 'back-of-a-truck' extraction facility was assessed. Extraction with hexane and ethanol were deemed unfeasible due to too long period of time required to process the amount of biomass produced by several small holder farmers. In other cases, the small scale plants are potentially feasible. In the case of HFC-134a a 0.4 m^3 compact plant has already been demonstrated.

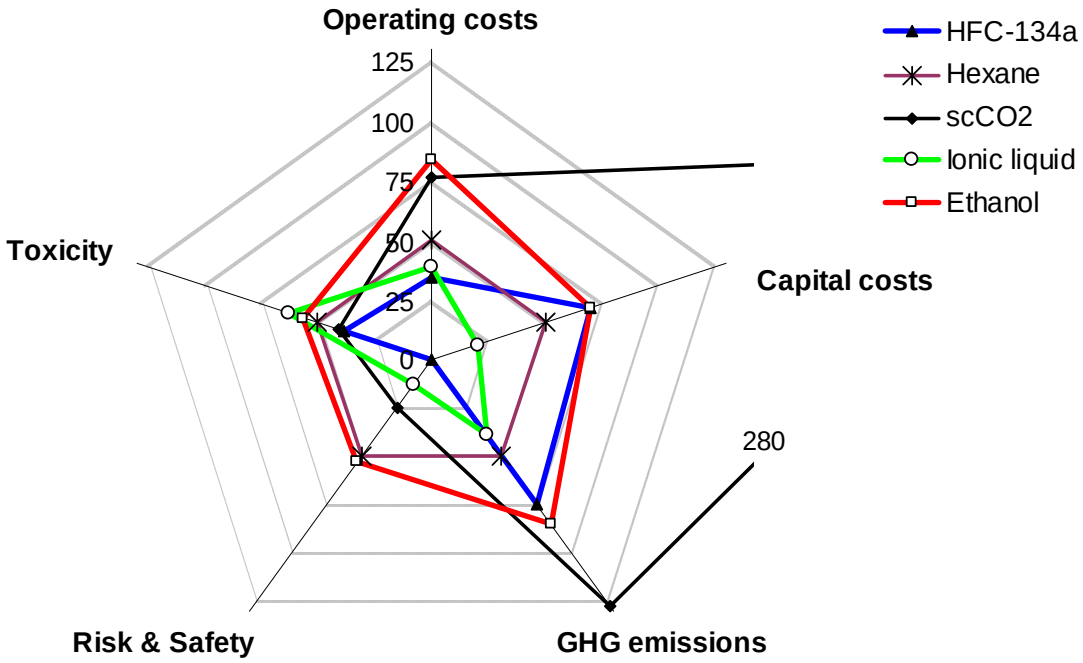


Figure 3. Multi objective comparison of extraction technologies. Base line case of hexane extraction is 50 on each axis. All parameters are scaled relative to hexane. An increase in performance, e.g. lower cost or risk, or toxicity, is given by lower values.

Conclusions and Recommendations

The main objectives of this study were to highlight new technologies which could be used for the extraction of artemisinin and to develop a bench marking procedure through which these new technologies could be compared to existing extraction methods e.g. hexane. In the case of ionic liquids extraction, which is an emerging technology, additional research had to be undertaken to provide sufficient data for this comparison to be made.

Whilst it is recognised that this study is not exhaustive its objectives have been achieved and most importantly, specific areas have been identified where additional support to technology providers, together with other research, will bring speedy and practical results for end users.

The results show that the new technologies, specifically HFC-134a and ionic liquids, have particular potential, being equal or better in extraction efficiency, extraction time, running and capital costs than hexane. Both technologies are also safer and are potentially more environmentally friendly (accepting that care has to be taken to ensure complete recycling and capture of the solvents).

HFC-134a is a proven technology for the extraction of a wide range of other natural products and it is recommended that support is provided to enable Artemisia field trails to be undertaken as soon as is possible.

Ionic Liquids show considerable promise and with additional research it is expected that a specific ionic liquid can be identified which combines both high efficiency and speed of extraction. The immediate problem of regeneration of the ionic liquid solvent and solvent recovery from the biomass is now being investigated. The use of ionic liquids in small scale, mobile, plants has been considered and their potential replacement for hexane in existing extraction facilities should be further investigated.

Mention must also be made of $scCO_2$ which exhibits high efficiency and speed of extraction. Its limitation is the higher capital and running costs, together with the need for experienced management due to the higher operating pressures, which will largely restrict it to larger, possibly multi-extraction, facilities.

A high level of interest has been expressed in the possibility of designing a viable, smaller, portable extraction facilities *e.g*. 20 tonne biomass throughput. The study shows that this is feasible and that a unit using HFC-134a has been operating for some time extracting other natural products. Potentially ionic liquids could also be suitable for a mobile unit. The limitation for all technologies, to date, is the long extraction time which would make such a unit non-viable. If the extraction time can be reduced, together with the possibility of undertaking an initial 'rough' primary extraction which can be 'refined' in a central process, a mobile unit could be viable. Recommended ongoing research includes work on reducing extraction and solvent recycling times and discussions with end users as to the future potential for small scale extraction.

In the course of the study a number of other significant problems/opportunities have been identified. These are listed below and action is being advised/implemented to resolve these issues:

1) Flexibility of extraction. Annex 1 details some of the pressures and limitations which extractors face when implementing an artemisinin only i.e. a mono-extraction facility, and the potential opportunities available through a multi-extraction facility approach. In particular is the long term potential for bio-refineries which use the whole plant biomass (including multi cropping).
   Action: Growers and extractors should investigate local projects involving bio-fuel and bio-refineries. Contact can also be made to the technology providers.
2) Post harvest treatment, drying and storage of leaves – very little work has been undertaken on this subject and variations in moisture content could have significant effects on the extraction process (which has still to be fully quantified for the new technologies). There is also evidence that artemisinin levels can increase during field 'wilting' prior to leaf stripping.
   Action: Trials with end users are now being planned.
3) There is a requirement for independent artemisinin testing facilities to undertake purity and specification tests. The facility will also help extractors refine their process to meet the process needs of their buyers.
   Action: Discussions are now being held with a number of institutions
4) The confidence level of new and existing growers and extractors is low due to the recent large variations in artemisinin prices and uncertainties over the future demand/supply of ACTs. Whilst there is an urgent 'market' need for more ACTs, the organisation and funding for their supply through the predominant public sector must be improved, clarified and speeded up. Until the access situation for ACTs is resolved growers, extractors and ACT manufacturers are unlikely to make the investments necessary to meet long term supply demands. This could also potentially result in the increase in supply of lower quality and counterfeit drugs.
   Action: Organisations including WHO/RBM, MMV, Gates Foundation, are now very aware of this situation and actions are being planned/implemented.

Comments and suggestions from readers of this report are requested and encouraged. Please contact Malcolm Cutler on mc@fscdev.com or fsc@onetel.com who will either answer them directly or pass them to the relevant source. Questions relating directly to a specific technology should be directed to the company(s) involved – see below for contacts.

TECHNOLOGY PROVIDERS - CONTACT DETAILS

Extraction with HFC-134a

Ineos Fluor
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Extraction with $scCO_2$

Essential Nutrition Ltd
Bank House
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Brough
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Extraction with Ionic Liquids

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Annex 1

Flexibility of Extraction Technologies

The Need for Flexibility:

Major producers/extractors may be able to make a financial and technical case for a one product extraction process *i.e.* 100% Artemisia, even with limited annual use. Smaller extractors will, however, almost certainly have to identify other crops which they can extract, during compatible seasons, with the same equipment. This ability to process multi crops could be particularly essential with small mobile extraction units.

All extraction technologies represent a major investment in both fixed and working capital. The project promoter or investor, be they a local company or an international pharmaceutical, need to ensure a return on this capital, which can only be achieved if there is an acceptable supply of raw material, matched by market demand. Outside of China and Vietnam Artemisia production is relatively new and whilst the market has considerable potential to grow, there are a number of factors (over and above local conditions, infrastructure etc) which a promoter/investor should investigate/ take into account, before committing to the project i.e. undertaking due diligence:

1) Artemisia is only just beginning to emerge as a viable crop and much has still to be learnt concerning seed varieties, crop husbandry and post harvest technology. In addition the regions where it is being grown vary greatly, with different climates, growing conditions and levels of infrastructure. Production is also widely spread over commercial farms and small holders.

With any new crop this situation is not uncommon and with time there will be greater understanding of production methods which, through "lessons learnt", trials and development will result in consistency and quality of production. In the meantime growers will most likely experience a steep learning curve.

2) At the present time the market is experiencing considerable uncertainty due, in part, to it being largely dependant on donor and aid funding (for the public sector). Predictions of present demand are therefore effected more by the availability of funds, and their being utilised efficiently, rather than the ultimate market need. This has already lead to under/over supply of artemisinin and consequent highs/lows in prices, a situation which does not provide farmers with the confidence that they can rely on a long term, viable and sustainable market for their Artemisia crop. Therefore farmers, especially commercial farmers, will be very cautious of investing in Artemisia, particularly if they can achieve greater stability and returns from other crops. Small holders may have a more short term view but if they are "persuaded" to grow a new crop, such as Artemisia and in a few years are told that there is no market or the margins are below other cash crops, they are unlikely to return when the market improves. They have been let down too many times before!

3) Much has also been recently published about the work surrounding synthetic or bioengineered endoperoxides, or other promising antimalarial drugs and vaccines. Whilst the wording often used by the press appears to promise that these advances will result in a total answer to the treatment of malaria, reality in likely to be very different. It is generally agreed that in the medium to long term they will play an important role, but the majority of treatments will still rely on naturally produced artemisinin. This, often confused, situation does little to help give confidence to both Artemisia growers and investors in artemisinin extraction facilities.

4) Buyers of artemisinin are presently limited to a small number of pharmaceutical companies. Sales through the public sector, financed through the Global Fund are presently limited to two artemisinin based products with only one, Coartem, being a widely prescribed ACT. Delays in approving other suppliers (and funds) are seriously restricting the expansion of Artemisia and the introduction of new extraction facilities. Local ACT production with GMP certified facilities (following 1st stage extraction which does not have to be GMP approved)), especially in Africa, could also potentially increase the number of ACTs dispersed.

5) Whilst, to date, there have been no reported cases of resistance to Artemisia based treatments it has to be considered in the long term, given the history of malaria parasite resistance to other compounds. There are however rumours of reduction in efficacy in regions where Artemisia based mono-therapies have been used for some years. It is for this reason that the WHO is now strongly resisting the continued supply of Artemisia based mono therapy treatments.

6) The increase in yields per hectare of dry leaf and artemisinin, though new crop husbandry and seed developments, could result in greater levels of financial returns per hectare, which would allow for mechanical harvesting/drying. This could then make Artemisia production viable for developing countries (economies of scale), to the detriment of production in Africa. Although this is at the moment only a hypothetical possibility, it could well become a reality in the future.

An example of a crop which 'bloomed' and then 'bust' is pyrethrum. In the 1960's East Africa was a major producer, but with the advent of cheaper synthetic products the market for the natural product crashed. Growers had to find other crops and extraction facilities shut, or in a few cases started to process other crops. Ironically one of these old facilities in Uganda is now reportedly being refurbished to extract artemisinin, whilst the market for natural pyrethrum has started to increase again.

It is for the above reasons that promoters/investors would well be advised to explore the possibility of multi use at an early stage in their planning.

It should also be stressed that the extraction facility is only one part of the production chain and therefore if multi use is planned, the growing of the necessary additional crops must be planned in good time to meet the demand of the factory and market.

What Other Products Can Be Considered?

The range of other potential products will largely be dictated by where the extraction facility is located i.e. the climatic, growing conditions and the market. Most recent Artemisia production has been in tropical climates, albeit at altitudes which allow temperate crops to be grown. The harvest times will also have to complement those of Artemisia. Many essential oil producers also work closely with the market on a contract basis, much in the same way as with artemisinin.

Suitable crops can probably be best divided into three categories:

1) Lower volume, high value herbs, spices, trees etc producing essential type oils e.g. Black Pepper, Paprika, Cinnamon, Fenugreek, Buchu, Pyrethrum, *prunus Africana*, roses
2) Higher volume crops such as tea, coffee, tobacco again producing essential oils
3) Commodity crops such as oil seed crops producing larger volumes of marketable oils for the food, consumer and energy industry.

Of particular interest is the development of the 'bio-refinery' principle where as large a proportion of the plant as possible is utilised to produce a range of compounds used as final products and as feedstock to energy and chemicals industries. With Artemisia this could include separation of the essential oils to produce camphor, artemisia acid and other high-value compounds. The remaining biomass *i.e*. stems and leaves after processing, could be used to produce bio-ethanol.

The concept of the **Biorefinery** has been proposed for a number of years. The basic idea is very simple; like oil, biofeedstocks contain a number of valuable building blocks. At present we cannot selectively separate these components in the way that oil is separated in the refining process into gasoline, kerosene, diesel, solvent fractions, naphta, light olefins, paraffins, etc. The reason why it is more difficult in the case of biofeedstocks, is due to the complexity of the molecules and large number of oxygen chemical functions, which present difficulty to conventional chemical approaches. At the same time there is a growing demand for biodiesel, bioethanol, natural drugs and fragrances. To increase sustainability of the biofeedstock processes it is necessary to attempt to utilise the plants more completely. For example, seed oil can be used to produce biodiesel, lubricants or solvents, whereas remaining cellulosic biomass can be used to produce bioethanol or energy in combined heat and power plants (CHP) and can also potentially be converted into chemical intermediaries.

In Africa there are presently a number of interesting developments concerning the production of Jatropha (*Jatropha integerrima* – also known as Peregrina), which is an evergreen shrub now being developed (in plantations) for the extraction of its oils, primarily for bio-fuels. However, the plant extracts are also used for medicinal purposes, as a dye and the oil seed cake as a fertiliser. A review of the Jatropha websites will provide considerable information on the growing of and uses for, the plant. Also detailed are companies such as D1 Oils (www.d1plc.com) who have developments in Africa.

Promoters and investors in new artemisinin extraction facilities therefore have a range of options which could directly effect the viability of their facility. In the case of a dedicated artemisinin supply chain it may be that the extraction of other products does not make sense in the short/medium term, but in the long term may minimise some of the risks of being dependent on a sole product/crop. Other, smaller, facilities may only be viable if they can be used for different extractions over the year.

The availability of new artemisinin extraction technologies could help increase the range of equipment/solvents which will enable promoters/investors to make the decision if, or when, to make this decision i.e. plan projects which will involve multi product extraction.

Technology Choices:

As this study has concentrated on artemisinin extraction the following is a brief indication as to the ranges of products extracted and their suitability for a multi extraction plant, based on artemisinin. Contact should be made with the manufacturers for more information or a review of existing literature for the established solvents.

Hexane and other solid-liquid, low pressure solvents:
Other than through steam distillation, this group of solvents are the most widely used in a very wide range of extractions. Considerable information on their use can be identified through existing literature, much of which can be gathered through the web.

The study has identified the limitations of the use of hexane (and similar solvents), particularly due to its poor safety and environmental properties. However, hexane has the ability to extract a wide range of compounds, but with limited selectivity, and has relatively low capital investment.

Supercritical CO_2:
scCO2 is an established technology, used to extract a wide range of products/compounds. As with hexane a literature review will identify the products successfully extracted by this technology *e.g.* essential oils, caffeine *etc*.

The viable application of $scCO_2$ technology to a multi-product plant will be very much dependent on the scales and costs of equipment. Due to the higher operating pressures and therefore the higher grade/quality of components required, resulting in higher costs, it will normally only be viable for a large scale plant. However, with improvements in technology and reduced manufacturing costs (for instance in the Far East or South Africa) $scCO_2$ technology could eventually be a viable option for a medium scale multi product extraction facility.

Ionic Liquids:
Ionic liquid is an emerging technology which to date has been trialled on only a limited number of products. However, with a large and growing number of ionic liquids being identified, the potential for 'tuning' the solvent to the specific extraction need is very attractive (although each new biomass may require a different solvent for optimal process). For more information and advice contact should be made with Bioniqs (www.bioniqs.com).

Hydroflourocarbons (HFCs):
HFC-134a has been used for a number of years to extract essential oils and other components from a wide range of herbs, spices and flowers. Little or no adjustment is required to switch between different biomasses although extraction/cycle times will need optimising for each product (as with hexane and $scCO_2$). Cleaning of the plant between different extractions can also be undertaken quickly and

efficiently. Annex II identifies products which have been successfully extracted using HFC-134a solvents. Further information can be obtained from:

- Ineos Fluor (www.ineosfluor.com)
- Phurua natural oils, Thailand (www.phurunaturaloils.com)
- Wilde Associates Ltd (www.wildeandcompany.co.uk)
- Bhubinder Khambay (Bhupinder.khambay@bbsrc.ac.uk)

Ethanol:
Ethanol is a very polar solvent and therefore has limited applicability. However, there are biofeedstock extraction processes based on ethanol. Its application is potentially effected by taxation regulations. Because of the natural limitation of the solvent, it is perhaps less suited towards multi-crop plants.

Annex II

The following is a list of products/materials which have reportedly been extracted successfully using HFC134a. Contact should be made with the companies listed for more information on these extractions and potential new product extraction.

Agar
Ajowain
Ambrette
Aniseed
Angelica seed
Artemisia Annua- Extraction and purification of artemisinin
Astaxanthin from shell fish & algi
Baies roses (Schinus terebinthifolius L.)
Basil – sweet (Ocimum basilicum spp)
Black Pepper (Piper nigrum)
Borage seed
Brandy flavour
Bear flavour
Buchu (Agathosma betulina)
Caffeine
Calendula- extraction and enhancement of isoharmentin 3-O glycoside content -
Cannabis- extraction and purification tetrahydrocannabinol and cannabidiol
Caraway
Cardamum seed (Elettaria cardomomum)
Celery seed
Champee oil
Chilli
Chocolate
Cinnamon- Extraction and fractionation (Cinnamomum zeylanicum)
Clove
Coffee oil
Coriander seed (Coriandrum sativum)
Cumin (Cuminum cyminum)
Echium seed
Evening Primrose seed
Fennel seed
Fenugreek
Frangipanier
Galanga(l) – (Alpinia galangal, Languas galangal (Linn) Stuntz))
Galbanum
Garlic
Ginger oil
Ginko Biloba (Leaf)
Ginseng Root
Green tea (Camellia sinensis)
Hops
Jasmine concrete/grandiflorum/sambac
Labdanum
Lemon peel
Lovage root/seed
Lycopene (from tomato skin)
Mace
Meadow foam
Melissa
Mogra
Mustard seed
Myrrh
Neem seed
Noot katone from citrus oils

Nutmeg
Oats (rolled)
Oolong tea (Camellia sp.)
Orange peel
Orris root (Iris pallida)
Paprika
Parsley
Patchouli (Pogostemon cablin (Blanco) benth)
Phormium Tenax
Pilea Microphylla
Pink pepper berry oil
Piperine
Pyrethrum flowers
Pyrethrum (olio resin)
Raisons
Rose oil
Rosemary- Extraction & enhancing rosmarinic & carnustic acid
Sage- S.Lavandulaefolia
S. prpurea
S.fruticosa
S.officinalis L
Sandal wood (Santalum album)
Saw Palmetto
Sea Onion (Siciliana Marittima)
Star Anise (Illicium verum)
St John's Wart
Tobacco Leaves- Extraction, purification of nicotine and adsorption onto ion-exchange polymer (chewing gum manufacture)
Tonka beans
Torreya Nucifera
Tuberose oil
Turmeric
Vanilla (Vanilla planifolia)
Vetiver
Wheat germ oil
Whiskey flavour
Wormwood
Zanthoxylum Americanum - *Genola*

Annex III

Table 1. Physico-Chemical Properties of Artemisinin

Parameter	Value
Molecular weight / $g \cdot mol^{-1}$	282.3
Melting point / ºC	156-157
Thermal stability in non-polar solvents / ºC	150
Solubility in water @ pH 7 / $g \cdot L^{-1}$	0.063
Solubility in water @ pH 7, 37 ºC / $g \cdot L^{-1}$	0.048*
Solubility in ethanol @ 21 ºC / $g \cdot L^{-1}$,	12
Solubility in ethyl acetate @ 20 ºC / $g \cdot L^{-1}$	100
Solubility in hexane @ 40 ºC / $g \cdot L^{-1}$	0.46
Solubility in hexane – ethyl acetate (5 %vol) / $g \cdot L^{-1}$	33
Solubility in N,N-dimethylethanolammonium octanoate / $g \cdot L^{-1}$	82
Solubility in bis(2-methoxyethyl)ammonium bis(trifluoromethylsulfonyl)imide / $g \cdot L^{-1}$	110
Octanol/water partitioning coefficient / log P	2.94

* Value for triclinic crystals obtained by recrystallisation from cyclohexane; recrystallisation from EtOH (50 %vol) solution yielded orthorhombic crystals with the lower and slower solubility in water.

Table 2. Artemisinin properties according to monograph

Artemisinin content	97.0-102.0 (by IR)	98.0-102.0 (by TLC)
T_M / °C	151 - 154	
$[\alpha]_D^{20^0\,C}$	+75 - +78°	10 $mg \cdot mL^{-1}$ solution in dehydrated ethanol
Loss on drying	< 5 $mg \cdot g^{-1}$	At 80 °C
Sulfated ash	< 1 $mg \cdot g^{-1}$	

Table 3. Typical artemisinin buyer specification

Appearance	Colourless to almost white crystalline powder
Purity by HPLC	NLT 99% (or greater)
Melting point / ºC	150 – 153
Loss on drying at 80 ºC	NMT 0.5% w/w
Residue on ignition	NMT 0.5% w/w
Optical rotation	+75 to +78

There are no named impurities.