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Energy in Commercially Available Ultra-Diluted Natural Cardiotropic Drug *Digitalis purpurea*: An UV Spectroscopic Study

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Abstract: Ultra diluted drugs have a statistically significant medicinal effect compare to placebo but it is still unknown how they work. Extract of foxglove plant, *Digitalis purpurea*, *Scrophulariaceae*, an effective cardiotropic natural product, is one such compound, which is used by almost all prevalent systems of medicine. As a natural cardiotropic drug it is presumed to have an energy component involved in producing the effect. Since the effect of *Digitalis purpurea* depends on concentration and dilution, it motivated us to carry out a systematic study to explore an energy entity in less than micro volume, commercially available drug *Digitalis purpurea*, diluted with aqueous ethanol. The diluent and cleaning agent used was free from any possible contaminant whatsoever. We report a preliminary UV spectroscopy analysis on the effect of serial ultra dilution in the medicinal plant extract *Digitalis purpurea*. Well-known technique of UV spectroscopy has been used for identification/characterization and analytical evaluation of the medicinally active ingredients of *Digitalis purpurea*. The experimental results, although preliminary, provide significant and reliable information showing standard UV spectral absorption peak within expected range. It also shows, the diluted out effect on the active component of the drug *Digitalis purpurea* and provides information to compute energy on serial dilution of drugs. It is found that 4.5 eV is required to dislodge the bond in the group. The eV content of a drug may become important issue for pharmacological and posological considerations.

Key words: Potentized medicine, ultra dilution, micro-volume, plant extract, ultra violet spectrum, energy

INTRODUCTION

For >2 centuries, aqueous ethanol ultra diluted (lower than micro-volume) medicine have been very much in vogue. Ultra dilution drug's billion dollar market and confidence of billions of people across the world in it are yet considered insufficient to give these ultra diluted drugs a satisfactory level of scientific and evidence-based status. Common methods have failed to detect the active drug substance in ultra diluted drug. The pharmacodynamics of the drugs producing biological changes is also unknown. Claims and counter claims have been made by researchers regarding detecting dynamic contents in ultra diluted drug, to name a few (Roberfroid *et al*., 1983; Cazin *et al*., 1987; Davenas *et al*., 1988; Maddox, 1988; Benveniste, 1993; Foreman *et al*., 1993; Louis, 2002; Hamman *et al*., 2003). Evidences, RCTs and the clinical records have elevated the rank of ultra diluted drug to be (in) significantly just more than a placebo. Selection of *Digitalis purpurea* was made because of its wide acceptance in treating heart ailments e.g., Heart failure, Dropsy etc by practitioners of modern medicine (Withering, 1775), as well as those of complementary, alternative (Hahneman, 1805) and other traditional types of medicine (1500 BC). Physical, chemical and biological characterizations are considered the benchmark for ultra diluted drug's to be at par with a scientific based system. In order to find evidence of presence of active ingredient in such a system of medicine (say, Homeopathy), a commonly used organic natural cardiotropic agent *Digitalis purpurea* was used. *Digitalis purpurea* is used by physicians of different disciplines in cases, which have contrary presentations. These contradictions have motivated us to carry out a systematic study to explore a detectable dynamic entity in ultra-diluted form of *Digitalis purpurea* available commercially, by UV spectroscopic fingerprints and characterization of effect of dilution with aqueous ethanol solvent on its ingredients. *Digitalis*, pharmacologically applicable for the entire group of its contents, is in use

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since long as arrow poison (1500 BC) and as a cosmetic. Preliminary UV spectroscopy analysis on the effect of ultra dilution on medicinal plant extract *Digitalis purpurea* have been *carried out* for identification, characterization and estimation of energy of the medicinally active ingredients of *Digitalis purpurea.* Recent reviews by Rao *et al.* (2007) have attempted to emphasize this aspect at length. However, they have come to a conclusion in their work that material science models and structural analysis using spectroscopic tools (Raman, UV-VIS) may not have reproducibility in medicine. However, Sharma has made preliminary investigation on the chemical environment of serially ultra diluted *Digitalis purpurea* using highly sensitive vibrational spectroscopy technique of FTIR and Raman analysis.

Out of about 30 known variable ingredients in *Digitalis purpurea* concentration, 6-7 constituents only are considered to have some medicinal value. The leaves of the second year's growth were used in collection, extraction and preparation[10] of ultra diluted *Digitalis purpurea* available commercially and produced as per the Indian homeopathic pharmacopoeia. The possibility in variation of result may come from the source as it is well-known that the concentration of ingredients of *Digitalis purpurea* varies with geographical conditions, season, age and even the part of the plant from where it is obtained.

The purified and active principles of *Digitalis purpurea* have been studied in material concentrations for their UV spectral recognition. This original research study presents characteristic recognition of UV absorption of commercially ultra diluted *Digitalis purpurea* as well as an analytical evaluation and standardization. Such findings are incorporated as they contribute toward consolidation of concept of ultra dilution. Drug and medicine have been used with the same meaning in the study.

Ultra dilution or serial dilution or potentized medicine: The saturated foxglove extract in aqueous ethanol (91.4% ethanol and glass double distilled water mixture) is represented as Θ or Q (mother tincture) in terms of Ultra-diluted medicine (say in Homeopathy). Dilution or potency '1' means one part of the medicine mixed in 99 parts of aqueous ethanol as diluents and succused. When the same process is repeated 30 times by adding one part of the previous potency in 99 parts of aqueous ethanol as diluents and succused, after each stage starting from first dilution for next 29 times, 30th potency is obtained and so on. These ultra diluted products are

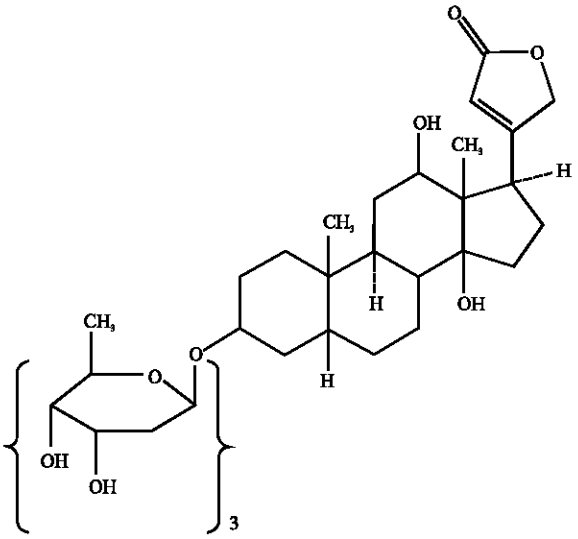

Fig. 1: Structure of digoxin

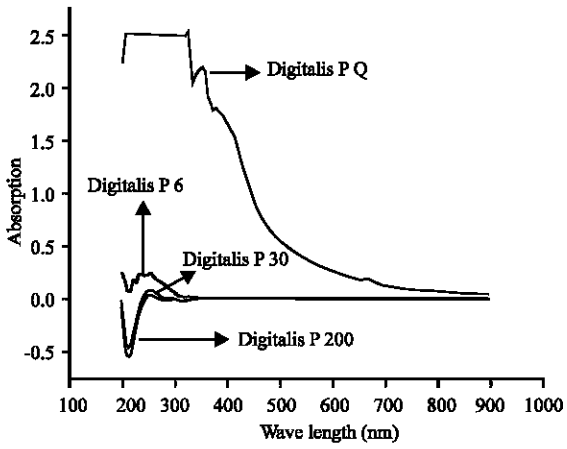

Fig. 2: Showing UV-VIS Spectra obtained for *Digitalis Purpurea* Q, 6, 30, 200

commercially available. Theoretically, thus dilution followed by succussion makes *Digitalis purpurea* `1` diluted to the level of 1×E-2, `30` has it 1E-60 and so on. Micro volume (10^{-6}) has been defined in the text as a concentration of one micro-liter or micro-gram of solute in one liter of solvent. Here any thing more diluted is considered as ultra-dilution.

Medicinally active ingredient of *Digitalis purpurea*, digoxin, have a steroid comprising a complex group of related genins distinguished by presence of OH (usually unsubstituted) at C 14 and additional OH group at C 12 and 16 (the presence and absence of OH) distinguishes the genins. An α, β-unsaturated lactone, a five member cyclic ester is present at C 17 with two conjugated double

bonds, as shown in Fig. 1. Other inclusions in *Digitalis purpurea* i.e., saponins, digitonin, tigonin and gitonin may have some antineoplastic and chemotherapeutic value. Hydrolysis of *Digitalis purpurea* produces three major aglycones or genins, all of which contain an α, β-unsaturated lactone ring in addition to other structural similarities. Both the C_{14} (β) hydroxyl group and the unsaturated lactone are essential to its activity as a drug. The effect of *Digitalis purpurea* on heart varies considerably depending on its composition and concentration, low dose and high dose (Williams and Lemke, 2002).

MATERIALS AND METHODS

Digitalis purpurea in potencies/ultra dilutions Θ, 6, 30 and 200 were procured from manufacturer M/s Hahnemann Publishing Company Pvt Ltd, Kolkata, India. The solvents/diluents are manufactured without any other inclusions such as acetone. In all experimental procedures the solvent used (even for rinsing of glassware etc) was ethanol. A thorough cleansing and rinsing with ethanol only, before and immediately after each reading, was carried out.

Background correction in the recorded spectrum has been carried out to nullify spurious signals/ noise as far as possible. The process of screening of the spectrum (range) of ultra dilutions was started with more diluted solutions to less diluted solutions.

Estimation of Nitrogen content in *Digitalis purpurea* was carried out using Vapodest 20-programmable distillation unit for Kjeldahl digestion from Gerhardt. UV spectroscope Spectrascan UV 2600 (Chemito) has been used for UV-VIS spectral analysis. The UV spectrums of the *Digitalis purpurea* θ, 6, 30 and 200 were analyzed. Collective spectrum obtained is given in Fig. 2. Calibration of the instrument was done after reading of each sample was taken. On an average, 20 spectral readings were taken for each sample. In the drug sample preparation for vibrational and UV-VIS spectral measurements, it was ensured that no such contamination with acetone occurs.

RESULTS

Analysis of *Digitalis purpurea* Θ/mL weighing 0.931 g showed presence of 0.0481% Nitrogen. As Nitrogen content is not a part of digoxin structure, emphasis has not been laid on it. Obtained absorption peaks are given in Table 1.

Table 1: Numerical view of the dilutions of *Digitalis*

Sample	Maximum absorption (nm)	Absorption intensity (a.u.)
Digit P Q	255	2.5 (saturated)
Digit P 6	255	0.247
Digit P 30	255	0.099
Digit P 200	255	0.053

UV: Spectra, Digit P Q = *Digitalis purpurea* θ (saturated concentration), Digit P 6 = *Digitalis purpurea* 6, Digit P 30 = *Digitalis purpurea* 30 and Digit P 200 = *Digitalis purpurea* 200

DISCUSSION

UV-VIS spectra: The theoretical maximum absorption in UV-VIS spectrum for a compound (Digitoxin) containing five membered cyclic α, β unsaturated lactone with a β substitution is as follows (in ethanol):

- A Five membered cyclic α, β-unsaturated ketone: 202 nm
- Acyloxy substitution (-OCOR): 25 nm
- β-substituted alkyl (including part of a cyclic ring): 24 nm (2×12 nm)

The above calculation predicts that the compound in ethanol medium absorbs at a maximum wave length 251 nm in UV-VIS spectrum. The recorded UV-VIS spectrum of the compound in ethanol solvent indicates more or less the same maximum absorption at around 255 nm due to conjugated $\pi \rightarrow \pi^*$ transition. May be, the small shift from 251-255 is considered as an impact of auxochrome group and/or the effect of the solvent ethanol (Mohan, 2004). The λ_{max} for polar compounds are usually shifted due to the effect of solvent, the λ_{max} for nonpolar compounds are usually not shifted in alcohol and hexane. Possibly the intramolecular effects involve the transfer of charge from one atom to another (the charge transfer spectra) (Dyer, 1984). It is known that the series of fine structure bands in the 230-270 mμ regions of the spectrum of benzene and those of most hydrocarbons are associated with vibrational effect on the $\pi \rightarrow \pi^*$ transitions. These fine-structure bands are particularly susceptible to solvent effects and are diminished and/or frequently destroyed in alcohol solution.

In the present study, the peak intensity decreases as the dilution of the sample increases throughout the series *Digitalis purpurea* θ> *Digitalis purpurea* 6> *Digitalis purpurea* 30> *Digitalis purpurea* 200, while the wavelength for maximum absorption remains the same. This hypochromic (i.e., the absorption intensity decreases) shift of the maximum absorptions can be explained from the Beer-Lamberts law (Mohan, 2004). According to this law:

$$\log (I_0/I) = A = \varepsilon Cl$$

Where:

A = The logarithmic ratio of I_0 (intensity of the incident light) and I (intensity of the transmitted light through the sample solution) is absorbance of the solution

ε, C, l = Molar absorptivity, concentration of solute and path length of the sample, respectively

Thus, due to dilution, as the concentration of the solute decreases in the solution, the absorption intensity also decreases when the other variables remain constant.

The details of spectra received are reproduced in Table 1.

Observed UV spectral absorptions for different potencies of *Digitalis purpurea* are given in Fig. 2.

Since, $\Delta E = h\ \eta/\lambda_{max}$ [E = energy; h = Plank's constant, η = velocity of light] = $(6.62\times10^{-34}$ JS$\times3\times10^{8}$ m $sec^{-1})/255\times10^{-9}$ m^{-1}= 4.5 eV.

Energy of 4.5 eV is required to dislodge the bonds of the group. It also appears from the above UV study that the compounds procured as *Digitalis purpurea* in all its dilutions have the same ingredients, which show no appreciable deviation in their chemical structure through UV spectroscopy because of the potentization of the medicine.

The sample mixture containing digitoxin, tigonin and desgalactotigonin absorbs in the same UV maximum wave length because in the other two compounds, tigonin and desgalactotigonin, only $n\rightarrow\sigma^*$ transition (at 180-250 nm) is possible for-OH group. The absorption corresponding to this transition is not detectable in the ordinary UV spectroscope due to absorption at around or above 200 nm. For recognition of these substances, vacuum UV instrument may be of use.

It may be noted that ingredients other than α, β-unsaturated ketone of *Digitalis purpurea* do not show any other specific UV absorption, so any change brought in because of dilution could not be ascertained. The UV absorption shows consistency throughout all the dilutions, signifying that no change occurs in the UV-perceptive chemistry of active ingredient aglycone. There is hypochromic shift because of decrease in absorption intensity which is a function of dilution effect. The UV-VIS spectrum of *Digitalis purpurea* in ultra-dilution gives clear indication of dilution effect in terms of changes in the absorbed intensity. The relationship holds well with experimental results as shown in Fig. 3. On dilution, occurrence of absorption band at wavelength 251 nm remains unaltered as shown in Fig. 2. Such a change in intensity profile possibly explains

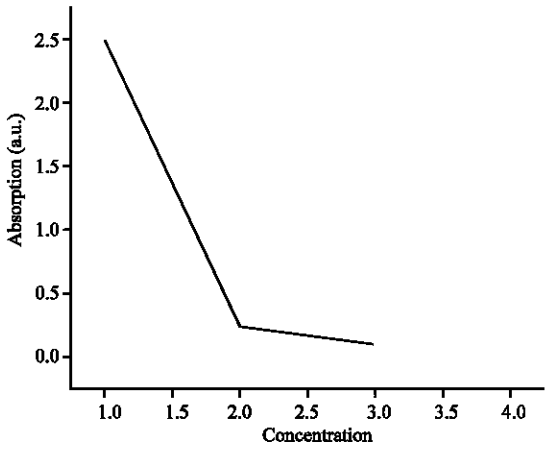

Fig. 3: Variation in intensity (absorption) maxima with changes in dilution concentration of *Digitalis purpurea*

pharmacological observations of the concentration/ serially diluted and succused (potency) drug. This analysis makes UV spectrometry one of the unilateral spectral tools for qualitative and quantitative evaluation of natural ultra diluted drugs.

Since, the conduction system of heart and nerve are energy driven, it may become important regulatory mechanism to fine tune the pharmacological application with it. It can also be possibly concluded that in the samples used for analysis of ultra diluted *Digitalis purpurea* in all its potencies, no compound as an impurity having UV absorption is detected.

Scope of further exploration: Exposure with proportionate energy of 4.5 eV in various dilutions of the *Digitalis purpurea* followed by UV spectroscopy may reveal changes in characteristics because of dislodged bonds of the group. The methodology can be adopted in standardization of ultra diluted/lower than micro volume drug substances and/or groups. The method can also be used to detect the UV-sensitive inclusions present, which in turn may be held responsible for the changes, if any produced by ultra diluted substances in chemical, physical and/or biological system. Categorization of ultra diluted materials in various solvents can be used for spectroscopic purposes to find out the relationship of substances at various energy levels. Relationship between energy and the quantum of diluted substance may be practically established and verified as a standardization tool. Such an analysis of a large number of medicinal plant extracts/substances can be used to prepare a standard data base for spectrally sensitive medical material.

CONCLUSION

The ultra diluted *Digitalis purpurea* gives UV spectral absorption peak within expected range. The active component of the drug *Digitalis purpurea* is diluted out. This method can be used for characterization and standardization of ultra diluted, UV-sensitive organic substances/components of the drug. The eV content of a drug may become important issue for pharmacological and posological considerations.

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REFERENCES

Benveniste, J., 1993. Frontier Perspectives 3: 13. DOI: 10.1038/366525a0.

Cazin, J.C., M. Cazin and J. Boiron, 1987. A study of effect of decimal and centesimal dilutions of Arsenic on the retention or mobilization of Arsenic in rats. Human Toxicol., 135: 315-320. DOI: 10.1177/096032718700600408.

Davenas, E. *et al.*, 1988. The Benveniste paper. Nature 333: 816-818. DOI: 10.1038/333816a0.

Dyer, R.J., 1984. Applications of absorption spectroscopy of organic compounds: In ultraviolet spectroscopy; Prentice Hall of India Pvt Ltd, Sixth Indian Reprint; pp: 10-11. ISBN: 0-87692-252-3.

Foreman, J.C. *et al.*, 1993. Nature, 336: 525. DOI: 10.1038/366525a0.

Hahneman, 1805. http://www.homoeopathicdoctor.com/hahneman.html.

Hamman, B., G. Koning and K.H. Lok, 2003. Homeopathically prepared gibberellic acid and barley seed germination. Homeopathy, 92 (3): 140-144. DOI: 10.1016/S1475-4916(03)00045-6.

Louis, R., 2002. Thermoluminescence of ultra-high dilutions of lithium chloride and sodium chloride Elsevier Science. Physica A., 323: 67-74. DOI: 10.1054/homp.1999.0259.

Mohan, J., 2004. Ultra violet spectroscopy: In Organic Spectroscopy Principles and application, Narosa Publishing House, N.Delhi, pp: 127. ISBN: 81-7319-566-8.

Rao, M.L., R. Roy, I.R. Bell and R. Hoover, 2007. The defining role of structure (including epitaxy) in the plausibility of homoeopathy. Homeopathy, 96: 175-182. DOI: 10.1016/j.homp.2007.03.009.

Roberfroid, M., J.C. Cazin and J.L. Gaborit, 1983. Action of Hahnemanian potencies upon artificially produced cancer in animals. Aspects of Res. Homeopathy, 1: 11-18. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=107841.

Williams, D.A. and T.L. Lemke, 2002. Foye's Principles of Medicinal Chemistry. 5th Edn. Lippincott Williams and Wilkins, International Student Edition, Philadelphia, pp: 499-501. ISBN: 0-7817-4211-0.

Withering, 1775. http://www.ch.ic.ac.uk/vchemlib/mim/bristol/digitalis/digitalis_text.htm.