Stachys officinalis (L.) Trev. St. Leon *Experimental Silver Nitrate Chromatography of ethanolic medicinal extracts*

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- Experimental Silver Nitrate Chromatography of ethanolic medicinal extracts

By

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I would like to thank Dr Anthony Deavin, MNIMH for his help in planning the technical aspects of the chromatography project and for sharing with me his experience, which has saved me many a wasted hour.

Introduction

This report presents the methodology, process and results of an experiment to produce Silver Nitrate chromatographs of medicinal ethanolic preparations of *Stachys officinalis*. The preparations were produced from the leaf of the plant, which was collected in six batches during a growing period of four months (16/3/2001 - 3/7/2001).

The aim of the experiment was to examine the pictomorphological properties of the different batch samples, represented through Silver Nitrate chromatography. By defining the general morphological characteristics across the samples, batch-specific variants of these characteristics were identified and described.

Method I:

The Herbal preparation

The herb (as leaf) was collected (3-5g) from the same plant at approximately two weekly intervals during the period 16th March to the 3rd July 2001. The plant was photographed prior to collection of the leaves (see appendix I). The collected leaves were then carefully washed with water to remove earth and dust. The leaves were then dried in a closed plastic container containing 50g desiccated silica-gel. The container was stored within a temperature controlled within the range of 15-20ºC during the drying process, which varied between 4-7 days depending upon the collection sample.

After drying, the leaves were stored in cellophane envelopes, which were placed in labelled paper bags, recording the collection date. These bags were then placed into long-term storage in a plastic container containing 10g silica gel, prior to making the preparations. The storage temperature was controlled within the range 2-4ºC,

The preparations were made as a 1:20 ethanolic maceration, using an ethanol concentration of 45% (reduced by demineralised water) (see Appendix II for quantities of marc/menstrum used).

The maceration was carried out in glass containers, which were stored in a darkened room controlled within the temperature range of 15-22ºC. The containers were agitated daily over a period of 7 days, prior to being pressed and filtered.

Pressing and filtration was carried out using disposable syringes, prepared by pressing glass wool into the bottom to a thickness of 5ml (the method is shown in appendix III). The pressed and filtered preparations were then placed glass bottles, with labels recording the collection, maceration start and final filtration dates (start and end of production phase). These were then stored at a temperature controlled within the range 2-5ºC.

During the entire production process, storage of the herb and subsequent preparations were carried out in the absence of light

Method II:

The Chromatography process

Material:

Filter paper: Munktell 5, 125mm diameter, class II
Silver Nitrate solution (0,05mol/l) from VWR International.

The filter papers used were marked with the corners of two quadrants using pencil. The quadrants were equivalent to 2 circumscribing circles of 30mm & 95mm diameter, having their centre in the centre of the filter papers (these marks were to be used as guides during the chromatography process).

The edge of each paper was marked with the date corresponding to the collection date of the preparation, which was to be tested on that paper.

The prepared chromatography papers were placed upon support cylinders constructed from 120g paper (100mm diameter, 55mm high). The preparation, corresponding to the data written on the periphery of the paper, was then applied to the centre of the paper by a 'drop system' (the method is shown in appendix III). Enough preparation was used to allow it to diffuse and 'wet' the filter papers up to the 30mm diameter marking (this was equivalent to two drops), after which, the papers were allowed to dry. This procedure was carried out three times to increase the preparation concentration (see the note below)

The papers were then further prepared by punching a 5,5mm hole in the centre of the paper.

Wicks were inserted into the hole in the filter papers. The wick was formed by rolling a piece of filter paper (50mm x 30mm) around a 5mm diameter wooden pin (along the papers 50mm axis).

The following stages were carried out concurrently
for the number of preparations to be tested:

The chromatography process was carried out in a specially constructed light-proof box containing a reservoir of distilled water (100ml) with an evaporating surface area of 176cm^2, that ensured a saturated vapour pressure within the box.

The prepared filter papers were placed in the box upon the support cylinders, arranged so that the wicks could be placed in reservoirs filled with enough of Silver Nitrate solution as to allow diffusion to continue to the 95mm diameter circumscribing circle marking (the method is shown in appendix III). The temperature within the box was controlled within the range 20-22ºC and the box was situated in a room with subdued light during the procedure.

The chromatographs were then removed from the light-proof box and allowed to dry and develop by exposure to daylight. The chromatagraphs were scanned into a computer within four hours of the completion of the chromatography process. This was done as to save the clarity of the chromatographs immediately following production (there is a slow deterioration of the chromatograph quality with time).

In addition to the preparations of the collected samples of *Stachys officinalis*, two control chromatographs were also produced concurrently for each batch. These were of the pure 45% ethanol used in the production of the *Stachys officinalis* preparations and a commercial extract of *Armoracea rusticana* (1:10, 45%), reduced to a 1:20 preparation. These controls were applied to the experiment in the same fashion as the *Stachys officinalis* extracts. The controls were made in order to judge the specificity of the pictomorphological properties of the *Stachys officinalis* preparations.

The entire chromatography procedure was carried out twice (batch run 1 & 2). Appendix IV shows the chromatagraphs produced.

Note.

Prior to the final experiment described above, comparative tests using a 0,05mol/liter (0,085%) and a 0,029mol/liter (0,05%) Silver Nitrate solution (see appendix V for dilution calculation) were carried out to evaluate the resultant chromatograph quality (see appendix VI). Similarly, the amount of preparation to be deposited on the paper was also evaluated for best results (see also appendix VI). In the final experiment, it was decided to use a 0,05mol/litre (0,85%) solution on papers that were impregnated three times.

Commentary on the process

Collection of the herb samples and preparation production

The samples were collected on each occasion from the same plant, which was situated on the Danish Island of Orø, at *Orø Urtehave*, (Orø Herb Garden) Denmark. This is a medicinal herbal garden open to the public, which grows herbs under organic conditions. The herbs were collected on the following dates:

16th March 2001
2nd May 2001
22nd May 2001
6th June 2001
19th June 2001
3rd July 2001

Irregularity of the collection intervals was due to practical problems regards scheduling of the project, the result being that the collection interval can be described as been 16 days ± 25% error. In all cases, collection of the samples was made between the hours of 12.00 and 14.00 for each sample. The washing and drying procedure was carried out in accordance with the prescribed method, immediately following collection.

Photographs were made prior to collection (see Appendix I), showing the physical growth and form of the plant at the time of collection.

The preparation production procedure was carried out in accordance with the description in the experiment method. The mathematical data for preparation production can be seen in appendix II.

Chromatography

The chromatography was carried out in a purpose built light-proof box, constructed from 10mm MDF board which was coated both internally and externally by a non absorbent lacquer. Provision was made for monitoring the temperature within the box during the chromatography process. Appendix III shows pictures of the apparatus and the 'set-up' used for the chromatography process.

Prior to carrying out the batch analysis on the preparations, it was necessary to experiment with differing amounts of preparation and with the concentration of Silver Nitrate, to establish the optimal combination. Appendix VI shows the results of these experiments.

Results

General pictomorphological characteristics and batch variants

As can be seen from the chromatagraphs (appendix V), there is a pictomorphological correlation between all the *Stachys betonica* preparations, the quality of which differs from the control pictomorphology.

The general pattern can be described, as having a distinct radial form comprised of four areas. These areas become clearer when seen in a contrast enhanced black and white picture (see appendix VII.).

Zone A, which I have termed the *clearance zone*, corresponds to the area defined by the initial application of the preparation. The characteristics of this zone are common throughout the batch in both batch runs, being an area lacking in any distinct morphological character.

Zone B, defines the area where the characteristic morphological form begins to emerge. This has a distinct denseness when compared to the general form. The extent of this zone varies throughout the chromatograph samples. However, measuring the height of this zone and taking the average for each preparation analysed, shows a gradual increase throughout the growing phase of the plant (see appendix VII).

The border between zone A and B also shows morphological changes throughout the range of preparations analysed. In the chromatographs of the earlier preparations, the border is characterise by *points of contrast*, these become less defined throughout the preparation batch and finally become very defuse in the two later chromatographs.

Zone C is the area that most characterises the general pictomorphological form. It is characterised by a *radiating* and *spreading* pattern arranged in a *spoke-like* fashion. This pattern is much more pronounced in the chromatographs corresponding to preparations from the 16th March and the 2nd May in both batch runs. These dates correspond to the beginning of the growing season characterised in the plant by petiolate leaf growth from the rhizome.

Zone D defines the area where the *spreading* of *spoke-like* radial pattern of zone C begin to *open up*. This pattern is not clearly visible in all the chromatographs, but is most apparent in those corresponding to preparations from: 16th march, 6th June and 19th June. These dates mark the beginning of the growing season and the beginning of the flowering stem growth for the plant.

Conclusion

The experiment revealed a pattern specific pictomorphology for the examined herb throughout the batch samples, when compared to the controls. The pattern also showed some morphological specificity corresponding to the collection dates and the growing phases of the plant in the preparations tested.

Interpretation of the morphological characteristics, and their specificity relating to the medicinal nature of the remedy under analysis would require further experimentation. Although some attempts have been made at interpreting chromatograhs (see Deavin 1992), they often rely on the application of a system of analysis, which relies on an abstract understanding. The development of a method of analysis unique to interpreting medicinal activity would necessitate a comparative analysis looking at the pictomorphological forms of different remedies with similar actions/ indications. By defining the specific qualities of the remedies that relate to pictomorphological form, it could be possible to develop a language of medicinal chromatograph interpretation.

16/3/2001

2/5/2001

22/5/2001

6/6/2001

19/6/2001

3/7/2001

Appendix I. *Stachys officinalis*
Photographed prior to collection

Sample collection date	Dried weight	Menstrum quantity* (45% ethanol). ml.	Preparation quantity after pressing & filtration. ml.	Waste menstrum % after pressing & filtration.
16th March 2001	400mg	8,00	5,30	33,75
2nd May 2001	400mg	8,00	5,50	31,25
22nd May 2001	400mg	8,00	5,70	28,75
6th June 2001	700mg	14,00	11,20	20,00
19th June 2001	500mg	10,00	8,00	20,00
3rd July 2001	800mg	16,00	13,00	18,75

**Note. Menstrum quantity = dried weight x 20.*

Appendix II. marc/menstrum quantities used in preparation production

Maceration process

Filtration process

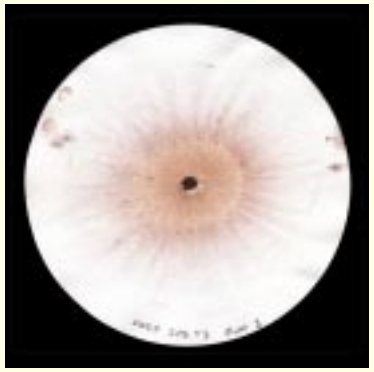
Completed preparations for chromatography, plus controls

Impregnation of filter paper

Chromatography dark-box

Appendix III. Preparation production and chromatography

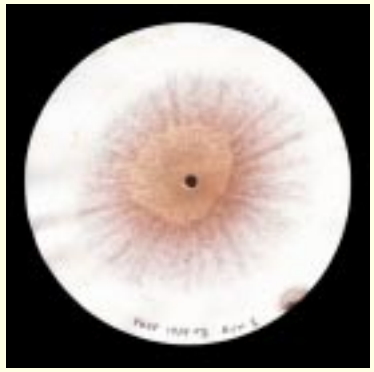
16/3/2001



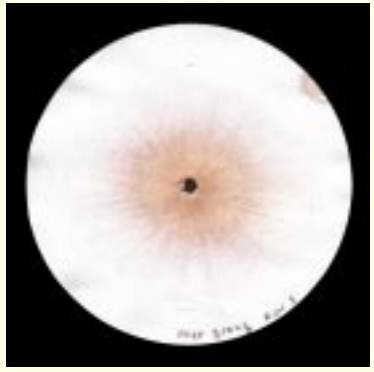
2/5/2001

22/5/2001

6/6/2001



19/6/2001



3/7/2001

Control chromatograph I

Ethanolic extract of
Armoracea rusticana 1:20: 45%

Control chromatograph II.

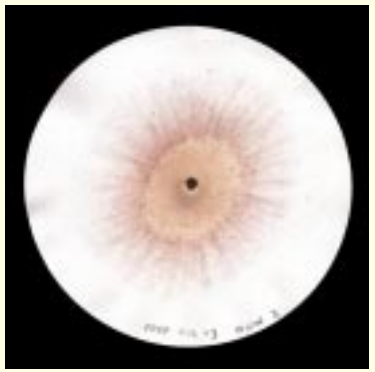
45% ethanol

Appendix IV, p. 1 of 2.
Chromatographs of the medicinal extracts (batch run 1)

16/3/2001

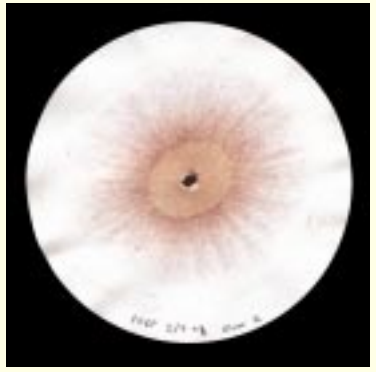
2/5/2001

22/5/2001



6/6/2001

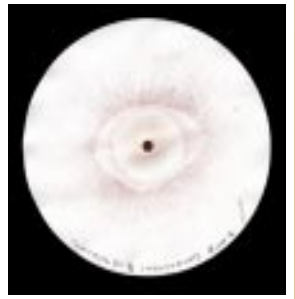
19/6/2001



3/7/2001

Control chromatograph I

Ethanolic extract of
Armoracea rusticana 1:20: 45%



Control chromatograph II.

45% ethanol

Appendix IV, p. 2 of 2.
Chromatographs of the medicinal extracts (batch run 2)

Silver Nitrate $AgNO_3$:

Molecular weight:	Ag	107,8682
	N	14.0067
	O	15.9994 x 3 = 47,9982
	Total:	169,8731

Therefore: 1 mol Silver Nitrate has a weight of 169,8731g

The original concentration of the Silver Nitrate solution = 0.05mol/L

0,05mol/litre solution contains: 169,8731g x 0,05
= 8,495g Silver Nitrate/litre

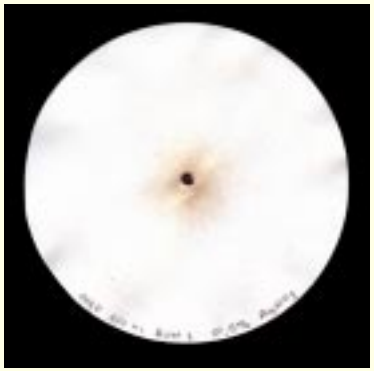
To create 100ml of a 0,5% solution

Ammount of Silver Nitrate in 100ml of a 0,5% solution = 500mg
Ammount of a 0,05mol/litre solution that contains 500mg Silver Nitrate:

$$= \frac{500\text{mg}}{(8{,}495\text{g}/1000\text{ml})} = 58{,}85\text{ml}$$

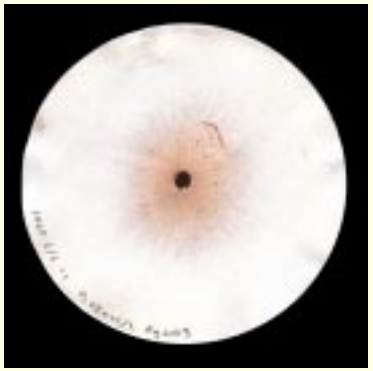
Therefor, to create a 0,5% solution. using an original 0.05mol/L solution:
Use 59ml of a 0,05mol/l Silver Nitrate solution, and add 41ml Demineralised water

Appendix V. Silver Nitrate dilution calculation

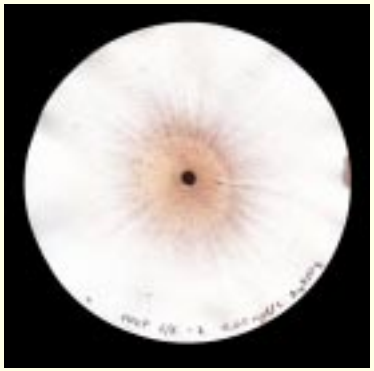


Preparation application: 1
0,05% Silver Nitrate

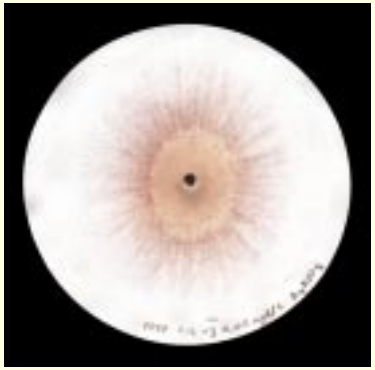
Preparation application: 2
0,05% Silver Nitrate



Preparation application: 1
0,085% Silver Nitrate

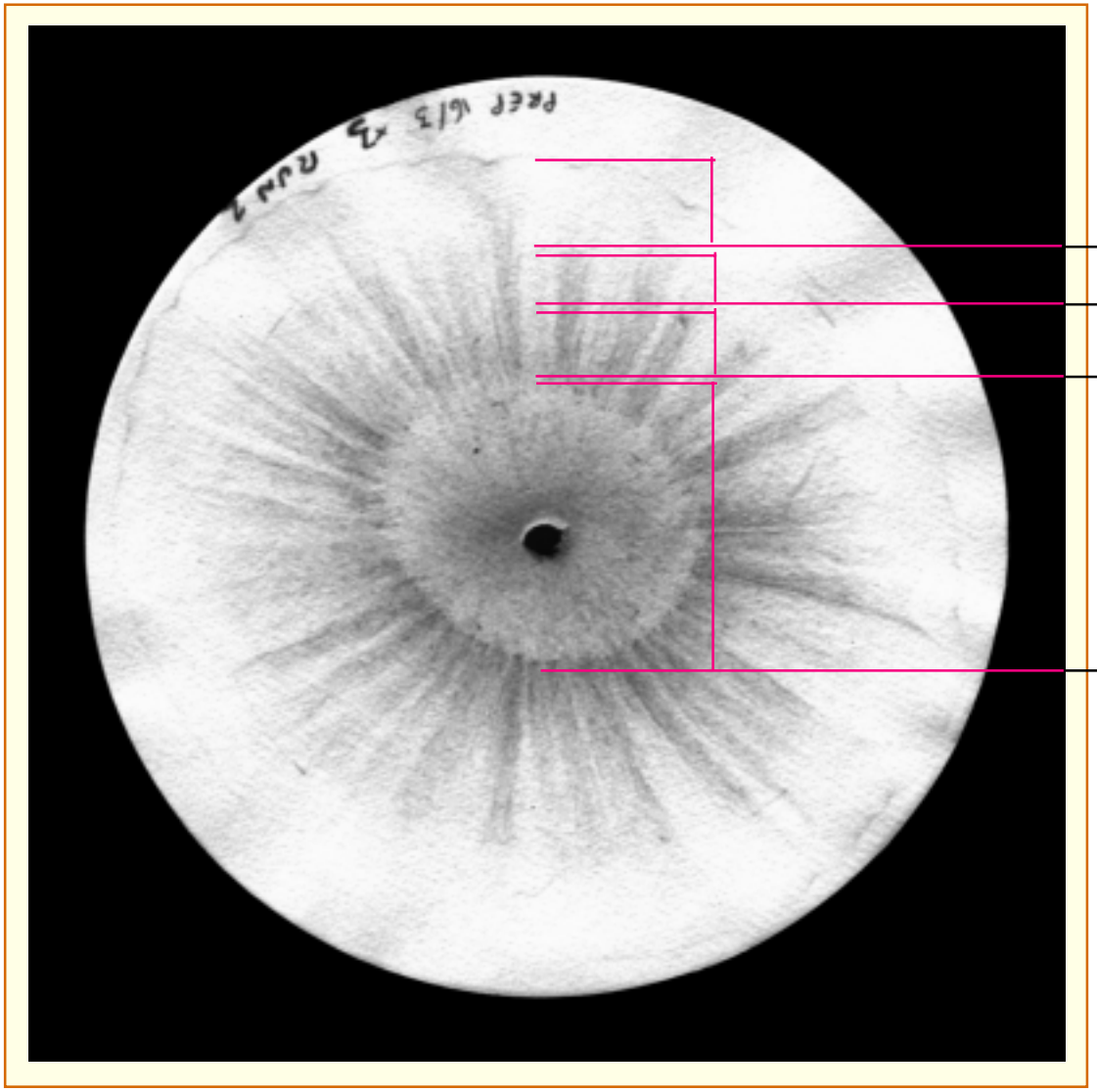


Preparation application: 2
0,085% Silver Nitrate



Preparation application: 3
0,085% Silver Nitrate

Appendix VI. Comparative trials for best concentration/solution combination



Zone A - height (in mm) from the chromatographs:

Preparation date	Run 1	Run 2	Average
16/3	**5**	**6**	**5,5**
2/5	**7**	**6**	**6,5**
22/5	**6**	**9**	**7,5**
6/6	**6**	**10**	**8**
19/6	**10**	**9**	**9,5**
3/7	**10**	**10**	**20**

Appendix VII. Pictomorphological characteristics

References and Bibliography

Andersen. J. O., 2001. *Development and application of the biocrystallization method*. Denmark: Biodynamic Research Association.

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Deavin. A., 1992. Energy and herbs: an investigation using the technique of sensitive crystallisation. *Complimentary Medical Research*. 6: 134-141.