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# When is Bilberry not Bilberry?

by Dr Reg Lehmann

During our usual market quality surveillance, MediHerb's Research and Development team of Dr Reg Lehmann and Dr Kerry Penman recently came across an example of an **adulterated** Bilberry standardised extract. Whilst they often encounter samples of very poor quality Bilberry, even they were surprised when confronted with this obvious example of faking a herbal extract by using an **added colouring agent**.

## Introduction

Bilberry (*Vaccinium myrtillus*) is a commonly used herbal product which contains anthocyanins – also called anthocyanosides. These are the blue/pink pigments responsible for the colour of ripe bilberries. The anthocyanins are also the markers used to assess the quality of Bilberry extracts. Up to 20 or more individual anthocyanins are present in Bilberry, so HPLC is not routinely used to quantify the levels of these in extracts. The *British Pharmacopoeia* (BP) specifies a spectrophotometric method of analysis, which essentially measures how much colour is present in the solution. This is similar to the method for the measurement of total hypericins in St Johns Wort. Such a non-specific test method leaves a material open to exploitation by unscrupulous suppliers and this is what we discovered in this instance. The sample was put through a three stage process of evaluation: Spectrophotometric testing; High Performance Thin Layer Chromatography (HPTLC); and Liquid Chromatography/Mass Spectroscopy (LC/MS).

## Spectrophotometric Testing

A sample of Bilberry dry extract standardised to 25% anthocyanins was received in the laboratory for analysis. When the sample was analysed by the spectrophotometric method the specification was met. In other words, the level of anthocyanins measured in the extract by this method came out at 25%. However, it was noticed that the extract was a slightly different colour than that of a normal Bilberry extract (refer to Figure 1). The sample was not the very bright pink colour as was expected, but was more of a cherry colour. Additionally when the pH of the sample was adjusted to >pH 10, this extract was a very odd purple colour (the colour of authentic Bilberry should be blue in this instance).

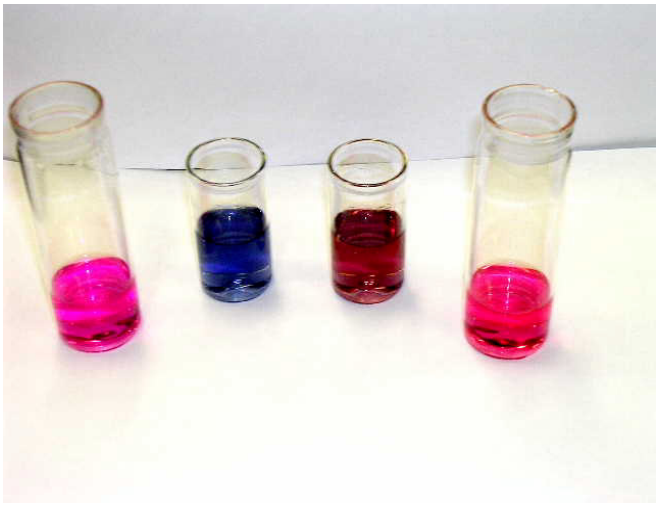


**Figure 1. Dilute solutions of Bilberry, left to right: very dilute authentic Bilberry, authentic Bilberry adjusted to pH>10, adulterated Bilberry adjusted to pH>10 and very dilute adulterated Bilberry.**

## HPTLC

The sample was then analysed by HPTLC which is used to separate a complex mixture into a fingerprint which can be used to qualitatively evaluate its components. When the Bilberry sample was compared to authentic Bilberry using HPTLC it was evident that sample was not a good match and in fact contained another ingredient which was responsible for the majority of the pink colour of the extract.

The sample was shown to contain only very small amounts of the anthocyanins which are found in authentic Bilberry. The majority of the pink colour was due to the band of colour at the base of the plate, which had only moved a very small amount. We suspect that this material may be a **food dye**. The only FDA approved red food dye (FD&C No 3 – **erythrosine**) was plated along with the pure colouring agent used in the extract (this was separated in our laboratory from the adulterated Bilberry sample). The erythrosine moved to the top of the plate, whereas the

colouring agent used in this sample stayed at the bottom. From this we could conclude that the colouring agent used **was not** the approved food dye erythrosine. **Hence the dye used could be potentially harmful and not fit for human consumption**.

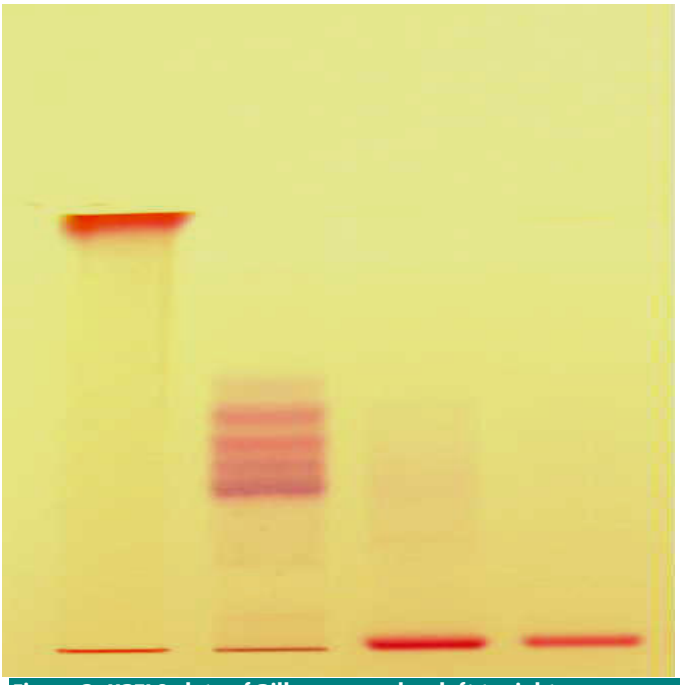


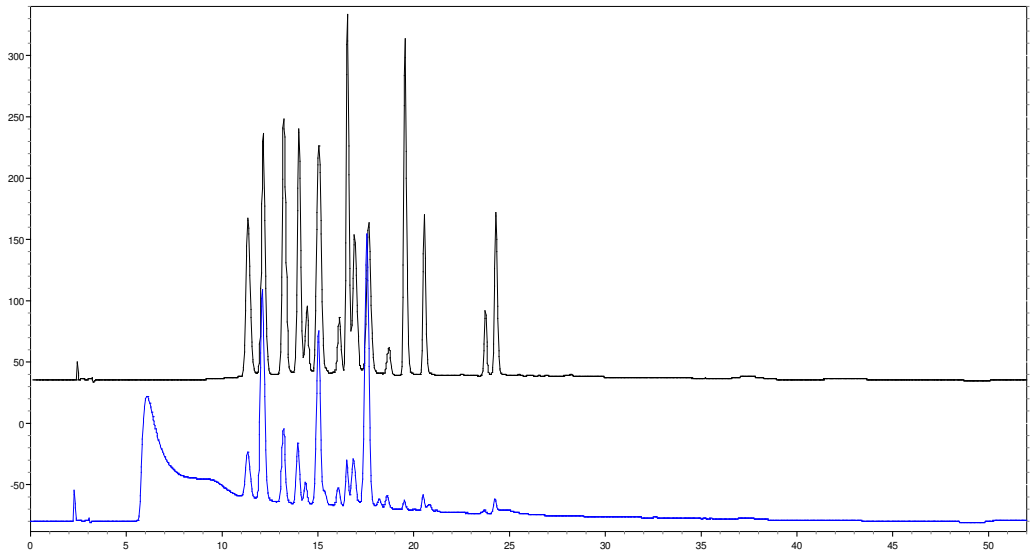
**Figure 2. HPTLC plate of Bilberry samples, left to right: erythrosine, authentic Bilberry, adulterated Bilberry, pure colouring agent.**

## LC/MS

For the development of the LC/MS method, we first ran the sample under the HPLC (High Performance Liquid Chromatography) method normally used by MediHerb to analyse all Bilberry samples. The traces obtained are provided in Figure 3. These traces show that authentic Bilberry is comprised of around **15 main** peaks, whereas the sample under investigation had **only 3 main** peaks and around 12 other minor peaks, plus an extra large "lump" in the chromatogram at 5 to 10 minutes retention time. It has previously been demonstrated that different species of Vaccinium have different HPLC profiles and this result therefore indicates that not only is this material adulterated with a colouring agent, the actual herbal part is also **unlikely** to be *Vaccinium myrtillus*.

The samples were next subjected to LC/MS which enables a mass spectrum for each of the components of the mixture to be obtained. This can assist to determine the actual identity of the peak in question. The mass spectra for the two Bilberry samples (Figures 4 and 5) were compared and it can be concluded that the large "lump" in the HPLC trace in the adulterated Bilberry is not structurally related to the anthocyanins and must be considered an adulterant. The ultra-violet visible light absorbance spectrum for the "lump" was also compared to that expected from anthocyanins (Figure 6) and it was shown that these also did not match.

**Figure 3. HPLC traces of Bilberry: top trace – authentic Bilberry, bottom trace - adulterated Bilberry.**



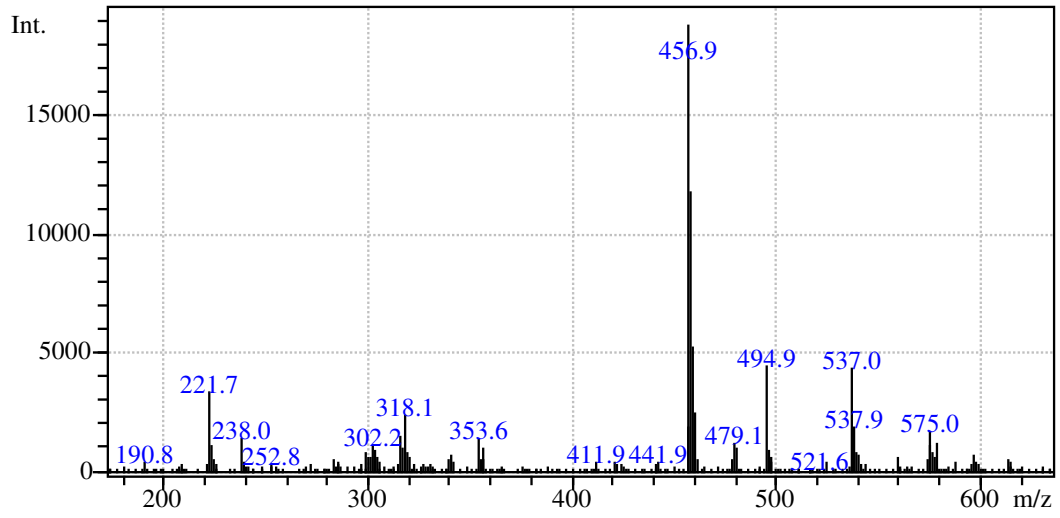



**Figure 4. Mass spectrum of adulterant in the sample of adulterated Bilberry.**

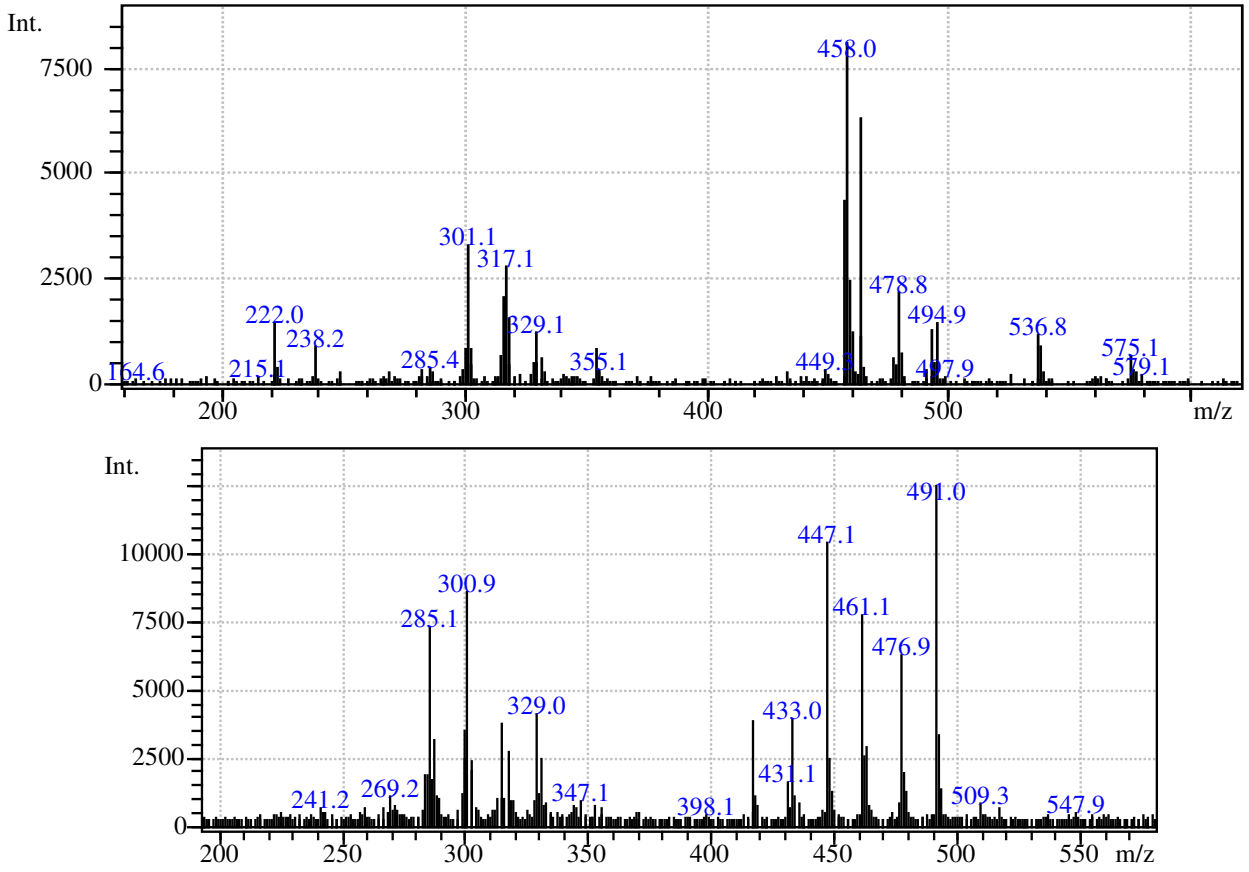



**Figure 5. Total mass spectrum of Bilberry samples: top – adulterated Bilberry, bottom – authentic Bilberry.**

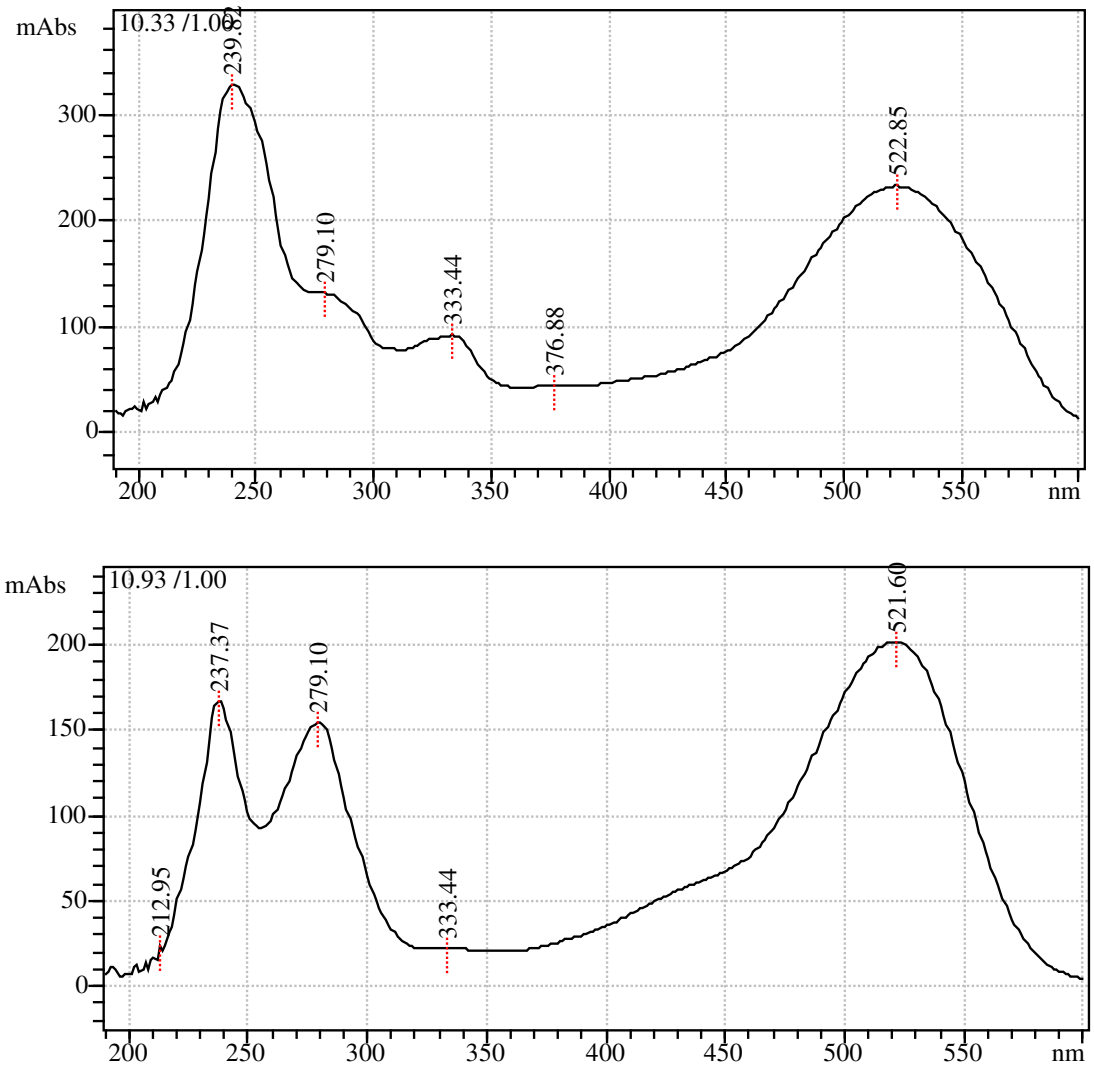



**Figure 6. Absorption spectrums recorded on LC/MS: top – large "lump" in adulterated Bilberry, bottom – typical anthocyanin from authentic Bilberry.**

## Conclusion

This analytical exercise illustrates two important issues in herbal quality. The first is, companies that buy extracts from suppliers and do not further test them, leave themselves open to be victims of adulteration, which could have serious health implications. At the very least the extract will not have the expected therapeutic activity. The second, perhaps more subtle issue, is that increasingly more sophisticated analytical methods are required to uncover the various complex quality issues for herbal ingredients. Specifically, a simple spectrophotometric method would not have detected the adulteration of this product.