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Detection of Biomarker: Resveratrol using HPTLC

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ABSTRACT: Cranberry, a natural occurring fruit, has been used for centuries in traditional medicine to promote urinary tract health. Cranberry extracts marketed as nutraceutical supplements and also for managing a variety of ailments, including microbial infections, metabolic disorders, type 2 diabetes and cancer.
Resveratrol (3, 4′, 5-trihydroxystilbene) is a naturally occurring phytoalexin found in Cranberry extract. As a phenolic compound, it contributes to the antioxidant potential. Resveratrol has been shown to regulate the metabolism of lipids, and to inhibit the oxidation of low-density lipoproteins and the aggregation of platelets. Furthermore, as phytoestrogen, resveratrol may provide cardiovascular protection. Additionally, this compound possesses anti-inflammatory and anticancer properties.
In light of this, the current study involved an attempt to quantify Resveratrol, an active marker, in two commercially available formulations of Cranberry extract. The mobile phase was optimized and Resveratrol bands were visualized on the TLC (Thin Layer Chromatography) plates.

KEYWORDS: Cranberry extract, Resveratrol, Standardization, HPTLC

INTRODUCTION

For many years, traditional medical systems (TMS) have served as the primary method of healthcare in many nations. Despite the current dominance of the Western scientific medical model, citizens and healthcare providers rely on and trust TMS in place of conventional, scientifically validated treatments. The Indian subcontinent has been employing herbal remedies ever since our rushi-munies discovered them many years ago. Although there are some differences between herbal & conventional pharmacological treatments, herbal medicine has gained popularity as a form of healthcare. The Ministry of Ayush, a ministry of the Government of India, is responsible for developing education, research and propagation of traditional medicine systems in India. Pharmacopoeia Commission for Indian Medicine and Homoeopathy (PCIM&H) and the Indian Pharmacopoeia Commission (IPC) have also signed a Memorandum of Understanding for the development of the "One Herb One Standard."[(1)]. The plant that was chosen for standardisation work was *Vaccinium oxycoccos* (common cranberry, northern cranberry or cranberry). *Oxycoccus palustris Pers*., popularly known as the European cranberry, is the small cranberry as shown in Fi [(2)]. Northeastern North America's wetlands and bogs are the natural habitat of the cranberry (genus Vaccinium). It is a member of the Ericaceae family, which includes roughly 125 genera and 3500 species and is sometimes known as the Heath or Heather family. The geographical distribution of the small cranberry, *V. oxycoccos*, occurs in forest areas in Europe, Asia, and

North America [3]. Cranberries have a life cycle of 16 months [3]. They develop on vines in sand, peat, gravel, and clay-filled substrates. Three months of frigid winter weather are needed for the cranberry growth season at 32°to 45°F to ensure flower set and fruit in spring. After three years, cranberry plants will start to produce fruits. Tamil Nadu, Kerala, Maharashtra, Karnataka, Andhra Pradesh, and West Bengal are among the Indian states that have cranberries [4]. In the past, cranberry fruits or leaves were used to treat diabetes of liver, stomach, and bladder diseases, among other illnesses. It can also be used on wounds. Nowadays, cranberry is used as a remedy for UTIs in women with recurrent infection [5]. Cranberries have a diverse and abundant phytochemical makeup; A-type procyanidins (PACs), anthocyanins, benzoic acid, and ursolic acid, in particular, flavan-3-ols, are among them and also contains polyphenols [6]. Interestingly, cranberry juice has a high content of polyphenols, one of which is an active marker known as resveratrol (RV). While both trans- and cis isomeric forms of RV exist naturally, their chemical structure primarily consists of two aromatic rings connected by a methylene bridge (Fig. 1), but the majority of the medicinal benefits have been ascribed to the trans-RV form [7]. Cis- and trans-isomers coexist in plants and in wine. The trans-isomer appears to be the more predominant and stable natural form [8]. Its several advantageous effects are the prevention of cardiovascular disease (CVD), antiangiogenic, immunomodulatory, antibacterial, neurological, diabetes and cancer. The aim of the current study was to check the presence of the active marker resveratrol in marketed preparations of cranberry extract. Using a novel, affordable, quick, and eco-friendly High Performance Thin Layer Chromatography [HPTLC] Method, we have attempted the quantitative determination of the Resveratrol content in marketed formulations of cranberry extract. HPTLC is said to be a better and efficient version of TLC. The high-throughput capability of HPTLC (to examine several samples at once) while only using a modest amount of mobile phase reduces both the time and cost of analysis.

MATERIALS AND METHODS

Chemicals and Reagents:

Resveratrol was purchased from Yucca Laboratories, Mumbai. All the other reagents used were of AR grade. HPTLC qualitative analysis was performed on silica gel 60 F_{254} (0.25 mm) plates (Merck, Darmstadt, Germany). Two marketed formulations- Cranpac capsules and BerryCran capsules were purchased from a local pharmacy store.

HPTLC Fingerprinting

Preparation of Standard Solution

Stock solution of resveratrol was prepared in methanol (20 µg/mL).

Sample Preparation

Both the marketed products were soft gel capsules and were analyzed in a manner given below:

Three soft gel capsules of each marketed product were cut to open and soaked overnight in methanol. The next day the solution was sonicated and filtered through Whatmann filter paper. The volume of the solution obtained was made up to 10 ml by methanol. About 1 ml of this solution was diluted to 10 ml with methanol. About 5 ml of the dilution was removed and volume was adjusted to 10 ml with methanol. The final diluted sample served as a test solution for HPTLC analysis. On two tracks 10 µl and 20 µl of test solutions were applied on the HPTLC plates.

Instrumentation and Chromatographic Conditions

HPTLC system included following:

Linomat V automatic sample applicator (CAMAG, Muttenz, Switzerland) for band spotting; 100-µL Hamilton (Bonaduz, Switzerland) – a glass syringe; twin trough TLC chamber (20 cm × 10 cm × 4 cm; CAMAG)**;** TLC Scanner 4 linked to winCATS software V. 4.06 (CAMAG); and HPTLC plates: 10 cm × 10 cm, having 0.2 mm layer of silica gel 60 F254 (E. Merck, Darmstadt, Germany).

Experimental conditions were the following: mobile phase consisted of chloroform: ethyl acetate: formic acid (8:3:0.5, *v/v/v*) with saturation time of 20 minutes; a detection wavelength of 308 nm; a slit dimension of 5.00 mm × 0.45 mm, a scanning speed of 10 mm/s and the source of radiation: deuterium lamp.

Resveratrol Detection

To detect the presence of resveratrol we applied the macerated sample solutions; 10 µL and 20 µL along with the 5to 25 µL of Resveratrol working standard on the adjoining tracks on HPTLC plate. The plate was developed under predetermined conditions and in the mobile phase described above. Further upon completion of the development plate was air dried and scanned at 308 nm.

To verify the presence of the marker compound i.e., resveratrol we performed a spectral scan of the peaks obtained at the same Rf value as standard Resveratrol on the track of test solution. Although the R*f* were matching, their spectral data did not.

RESULT

Resveratrol is reported to be present in cranberry extract. The cranberry extract is available in different dosage forms such as capsules and syrups. In the reported experiment, we performed HPTLC analysis to detect presence of active marker i.e. resveratrol in the marketed formulations of cranberry. We developed the simple and robust HPTLC method to detect resveratrol. We could detect the presence of peaks in samples at the same Rf value of standard resveratrol. Further, when we scanned the plate for spectra of both the standard and sample band, we observed that spectra did not match. Hence, from the current analysis it is seen that although Rf value is similar, spectra did not match. Therefore, we conclude that presence or absence of resveratrol in the marketed formulations of cranberry extract should be concluded only after confirming spectral match with standard marker.

DISCUSSION

The presence of active ingredient in herbal preparations is necessary to have desired pharmacological effects. Herbal drug standardization is necessary to ensure the presence of active phytoconstituent. HPTLC is the simple yet effective method to determine the content of phytoconstituents in raw herbal material as well as formulations. The negligible amount or absence of active phytoconstituent in the herbal preparations may not give desired therapeutic effects, therefore standardization is important to ensure the quality of material.

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Fig.1- Cranberry Plant

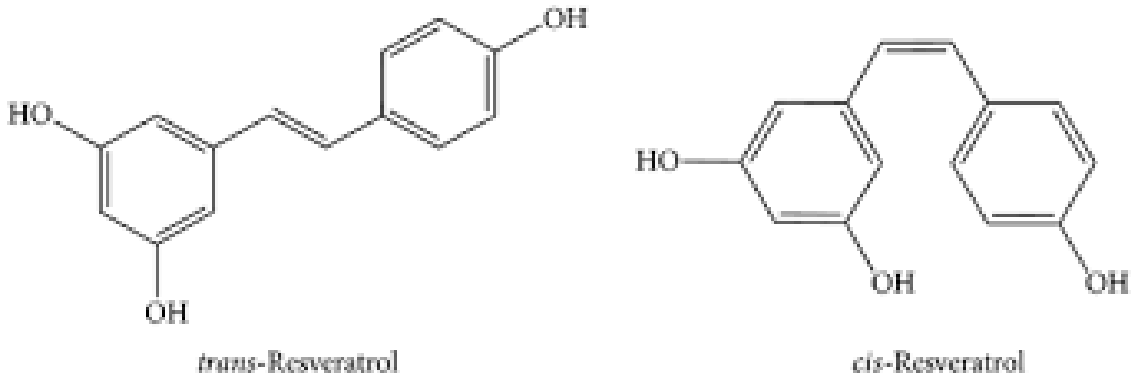

Fig 2- Structure of Resveratrol

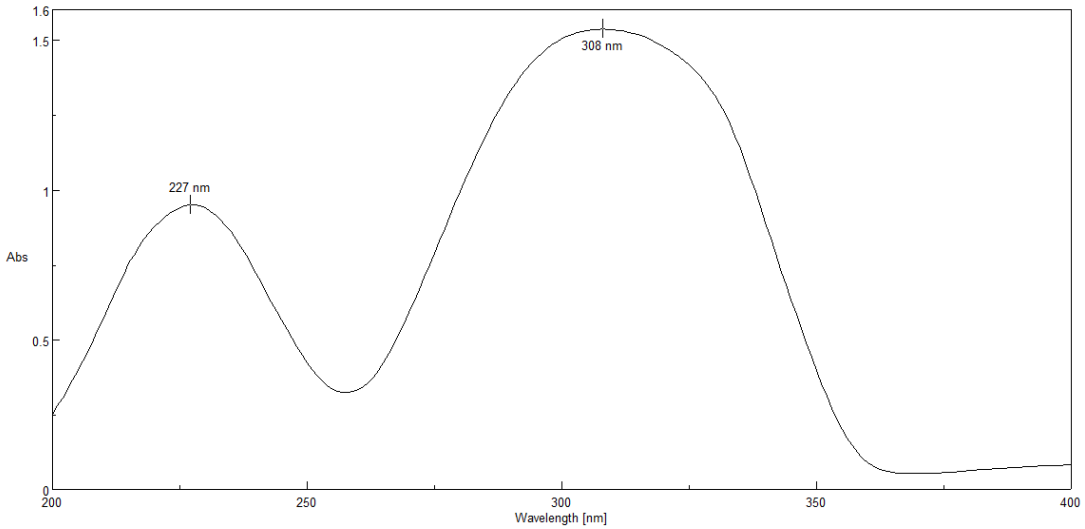

Fig 3- UV Absorbance Spectrum of standard Resveratrol (10 µg/ml)

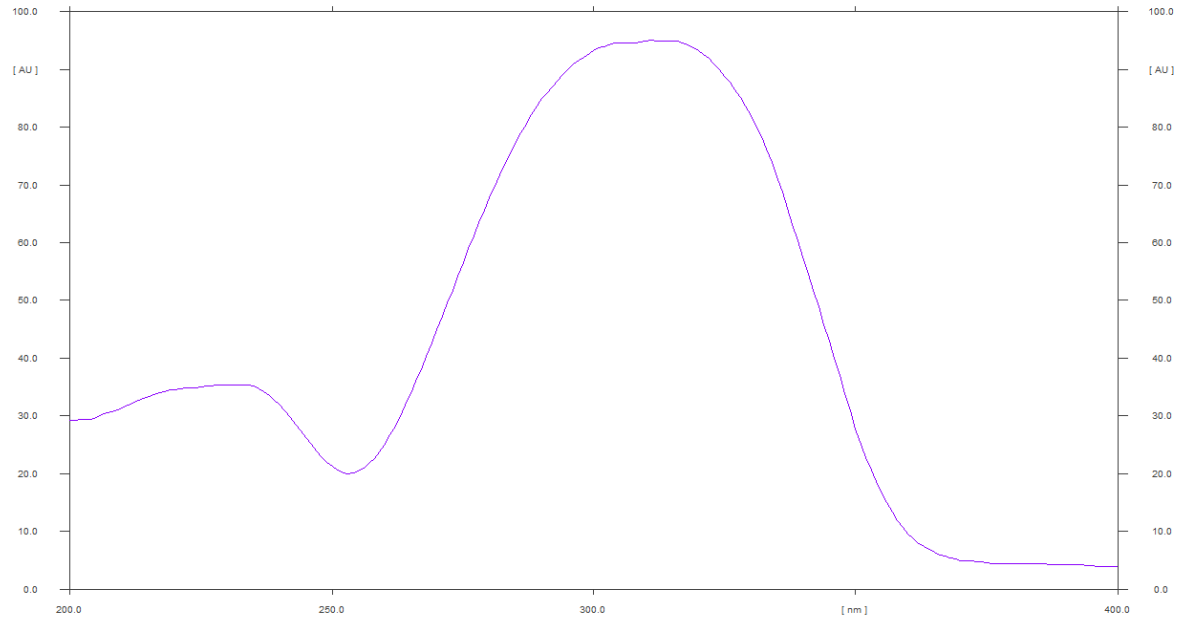

Fig 4- UV Spectrum of standard Resveratrol band from TLC plate

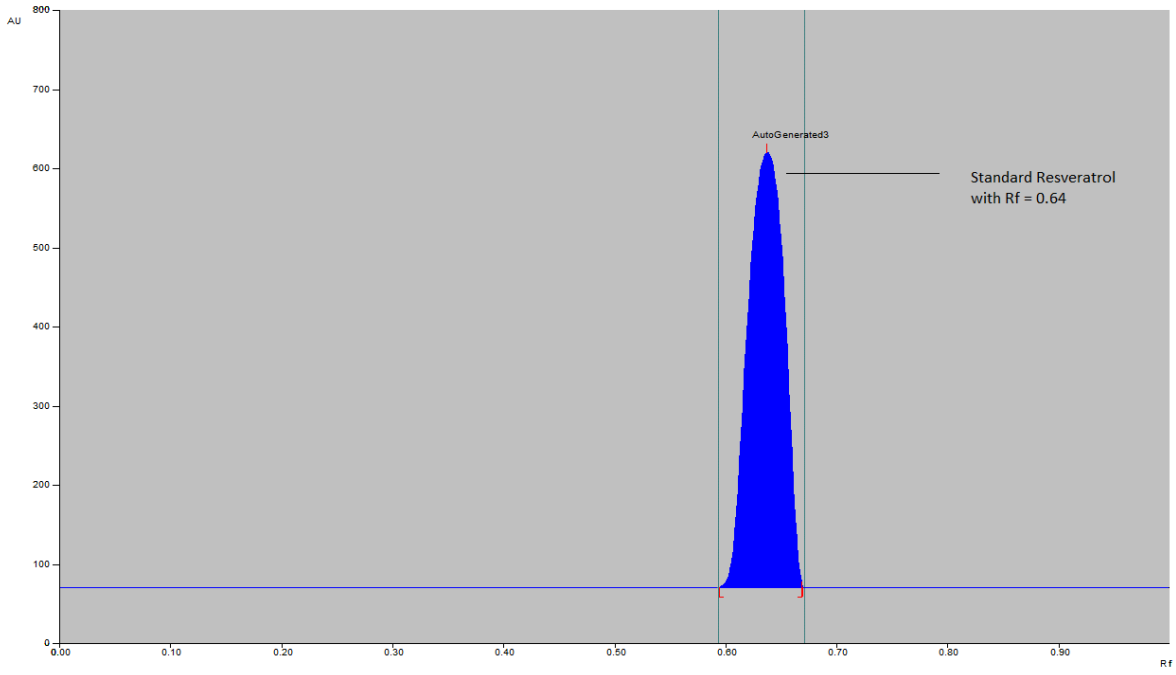

Fig 5- Densitogram of standard Resveratrol

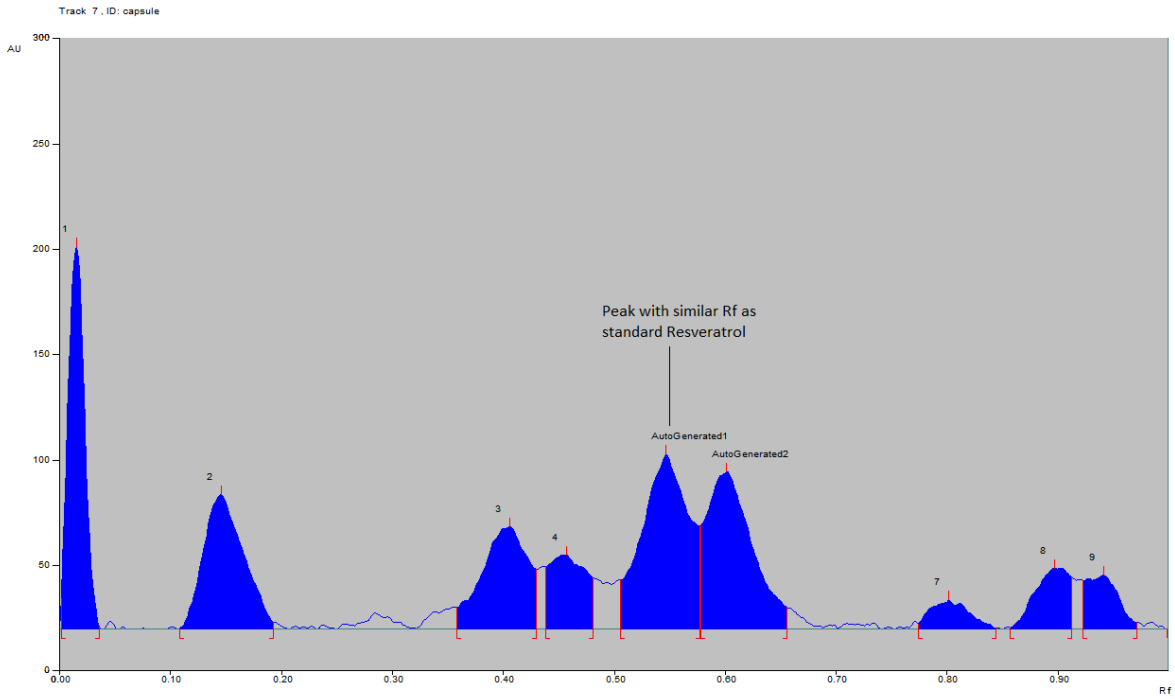

Fig 6 - Densitogram of Cranpac Capsule (track 7)

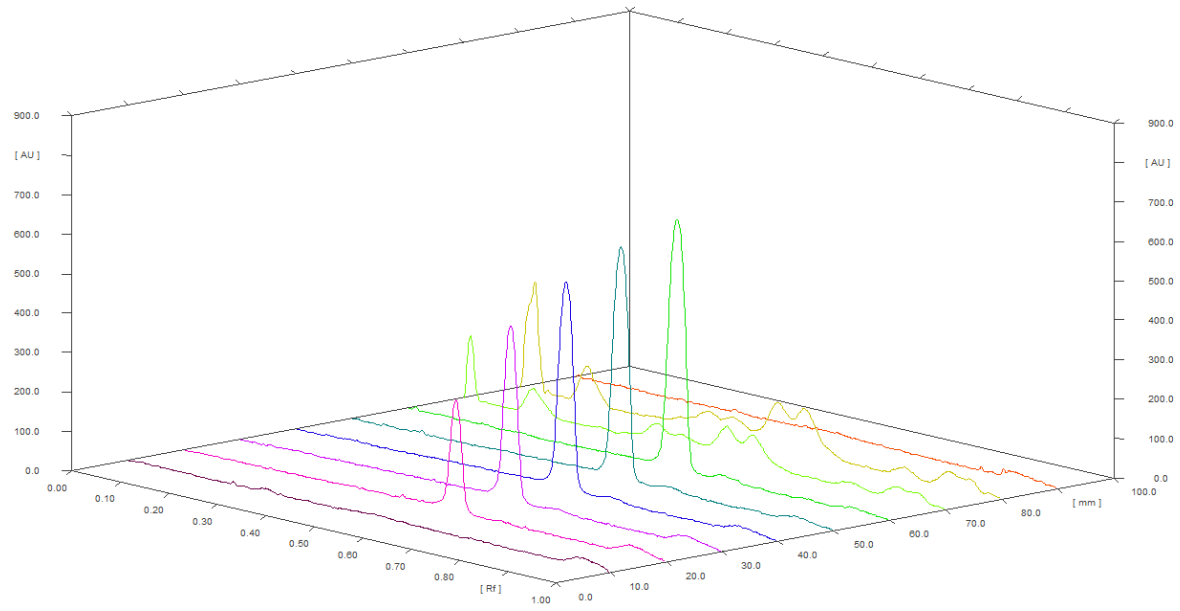

Fig. 7- Track 1,9 blank; Track 2-6 Resveratrol linearity; Track 7-8 of marketed product (Cranpac Capsule)

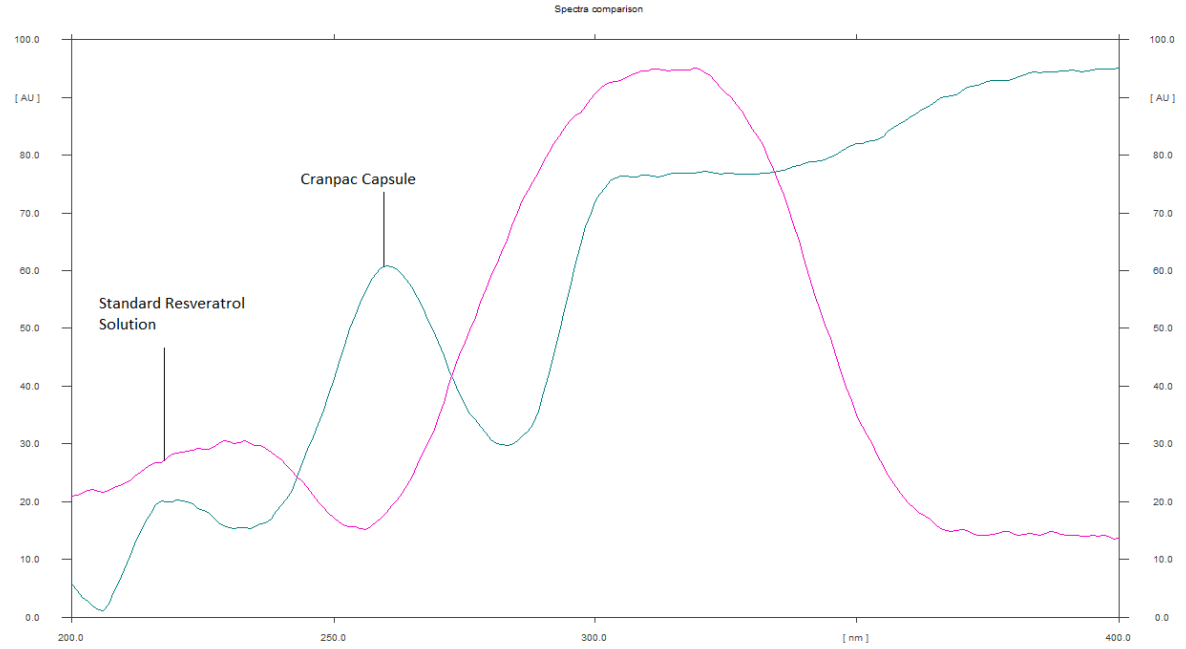

Fig 8- Overlay of UV spectra of resveratrol standard (track 6) and Cranpac capsule (track 7)

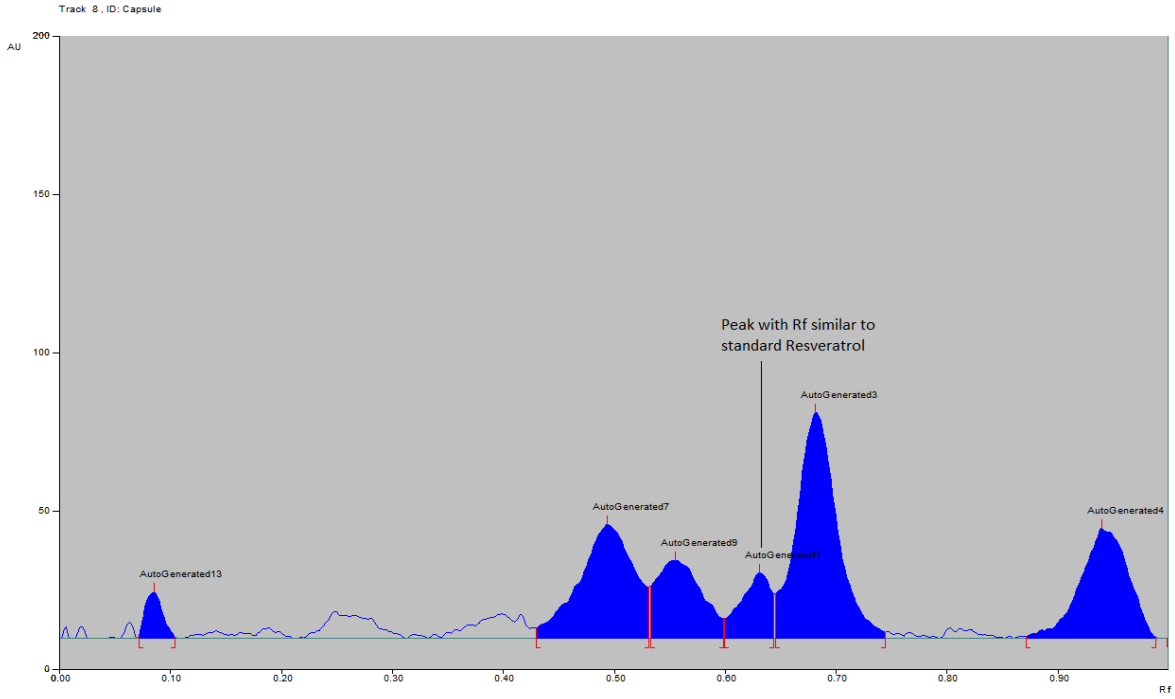

Fig 9- Densitogram of BerryCran Capsule (track 8)

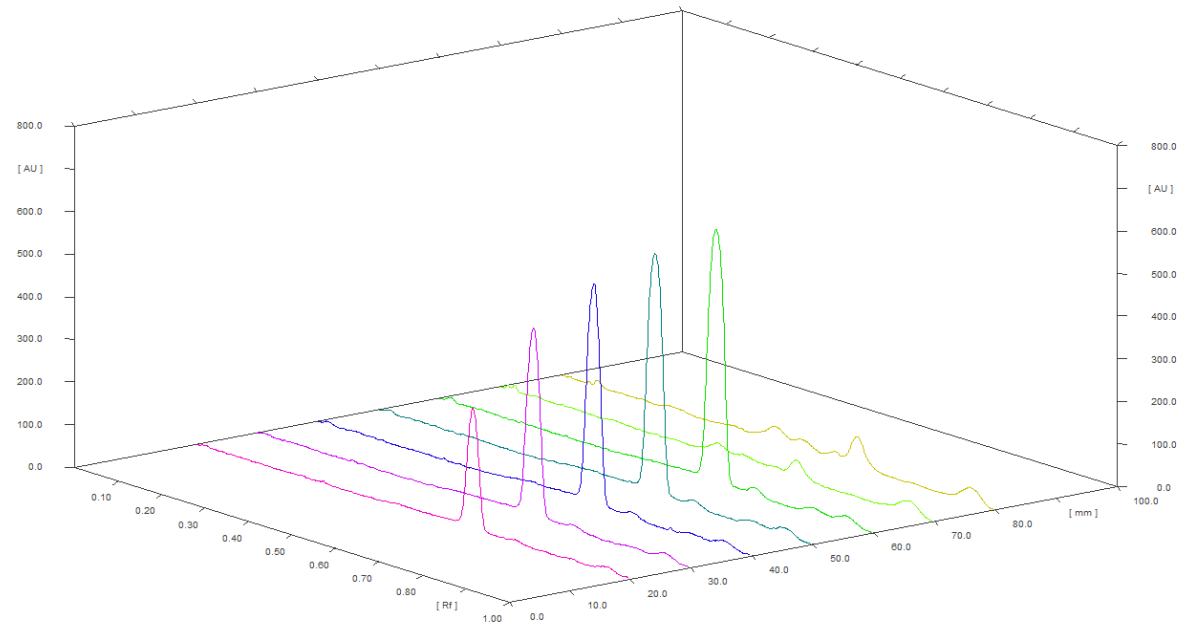

Fig. 10- Track 1,9 of blank; Track 2-6 of Resveratrol linearity; Track 7-8 of marketed products (BerryCran Capsule)

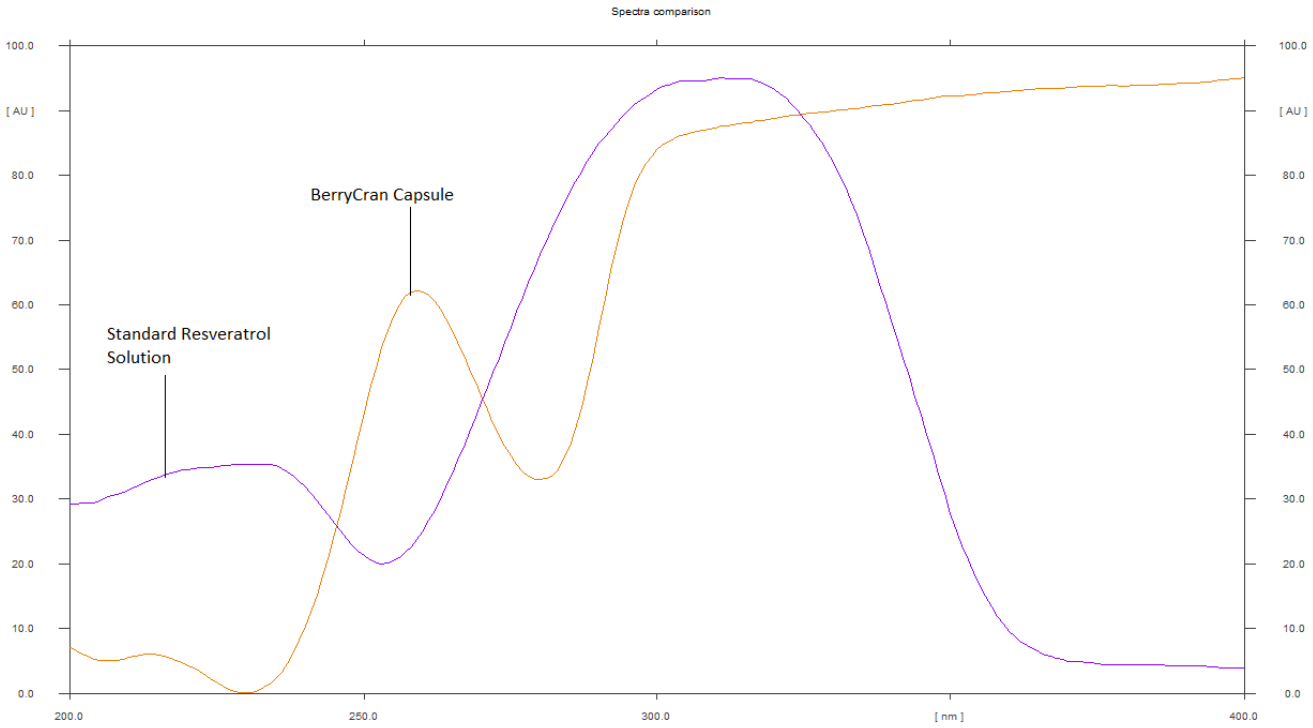

Fig 11- Overlay of UV spectra of resveratrol (track 5) and BerryCran capsules (track 7)