

## Sarcosine as a Stage Dependant Metabolomic Marker to Detect Prostate Cancer by Using HPTLC

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**Abstract:** Biomarker approach is to improve screening, detection and the diagnosis of early-stage prostate cancers. Over diagnosis and false positives present significant clinical problems in the prevention and treatment of cancer. Therefore, there are unmet needs to more accurately identify early-stage cancers and distinguish lesions that are life threatening from those that are not. Sarcosine was used as a model analyte in this study due to its potential for serving as a prostate cancer metabolomic marker.

The normal concentration of Sarcosine in the blood is below limit of detection and remains undetermined (Bellon, 1984). Therefore, bio-fluid urine was selected for the analysis purpose. Based on the literature, initially standardization of Sarcosine was carried out. Thereafter, prostate cancer patients' urine samples of various stages are analyzed in order to detect and analyze prevalence of biomarker with progression of the disease.

**Keywords:** Metabolomics, prostate cancer, Sarcosine, HPTLC.

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### I. Introduction:

Prostate cancer (CaP) is the most common type of tumors disease in men. Early diagnosis of cancer of the prostate is very important, because the sooner the cancer is detected, the better it is treated. According to the fact, there is great interest in the finding of new markers including amino acids, proteins or nucleic acids. Prostate specific antigen (PSA) is commonly used and is the most important biomarker of CaP. This marker can only be detected in blood and its sensitivity is approximately 80%. Moreover, early stages cannot be diagnosed using this protein. Currently, there does not exist a test for diagnosis of early stages of prostate cancer. This fact motivates us to find markers sensitive to the early stages of CaP, which are easily detected in bio-fluid urine. The objective of this project is to stimulate and support biomarker research to develop, optimize, and clinically validate novel Marker for early detection marker.

- To test developed Cost effective method to detect metabolomic marker in prostate cancer patient's bio fluid, urine.
- To test developed method to detect marker level in different stage of cancer patient's urine.
- To validate the developed assay to detect marker for cancer patients.

### II. Materials And Methods

A HPTLC system (CAMAG, Switzerland) consists of a sample applicator, Linomat 5 and scanner, CAMAG TLC 3 scanner. The sample analysis was carried out using stationary phase, silica gel 60 F254, 20x10 cm TLC plate (Merck). The reference standard compound of Sarcosine was obtained from Sigma-Aldrich (S7672-10MG). Methanol, Chloroform, Toluene, Acetone and diethyl amine (4:2:2:1.5:0.5) were used of AR Grade for solvent system (Bellon,1984).

#### 1. Preparation of Sarcosine stock solution:

The standard Sarcosine solution was prepared by dissolving 10 mg of Sarcosine in 1 ml of double distilled water which gives the concentration of 10000 µg/ml. Different concentrations of 140 ng, 250 ng, 500ng, 2µg, 4µg were prepared from standard solutions (Burton,2012).

#### 2. Experimental conditions:

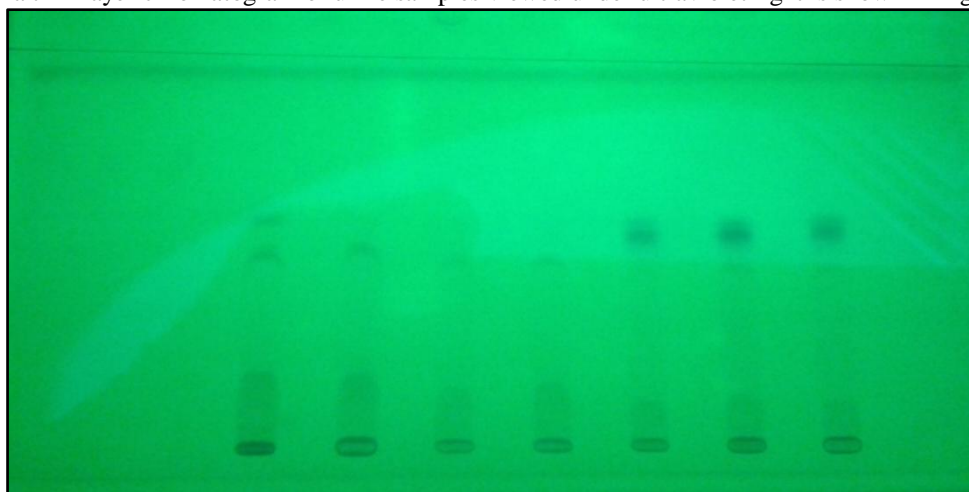
Initially, impurities on the silica gel 60 F254, 20x10 cm TLC plates (Merck) removed by pre-development process using methanol (AR grade) and air dried at room temperature. The HPTLC analysis was carried out by applying band of 6mm of each test samples and standard samples. After that plates were developed in twin trough glass (CAMAG) chamber of size 20x10 cm which was prior saturated with solvent system chloroform: acetone: toluene: methanol: diethylamine (AR grade) (4:2:2:1.5:0.5) for atleast 20 minutes and then plate was developed by ascending method. The other parameters like position of solvent front 50mm, slit dimension 5.00 x 0.45 mm and detection wavelength 254 nm were used for present study.

### 3. Prostate cancer patient's urine sample collection:

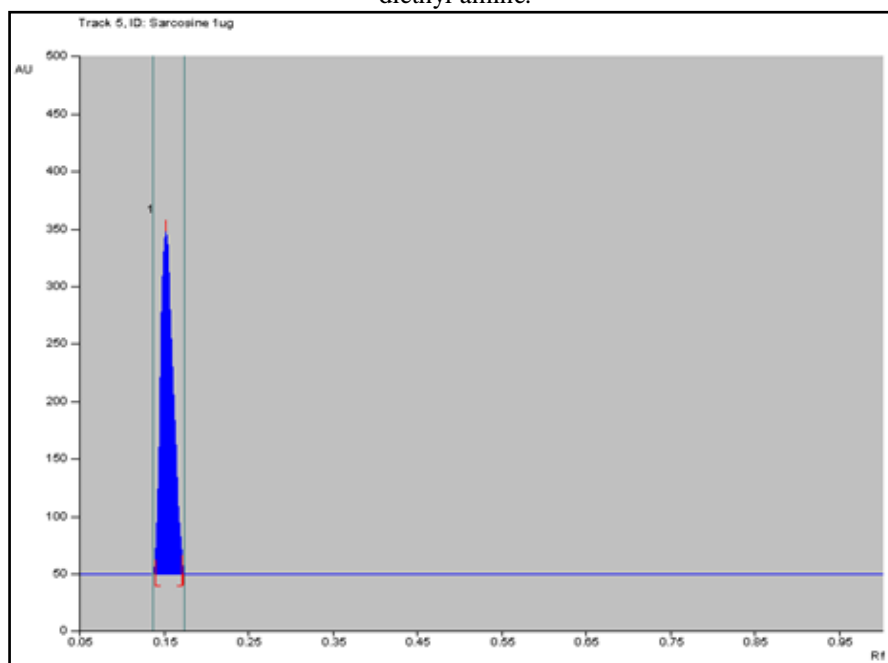
The samples were collected with prior consent of patients according to recent ICMR-DBT guidelines.

### III. Results And Discussion

A photo of a thin-layer chromatogram of urine samples viewed under ultraviolet light is shown in Fig. 1.



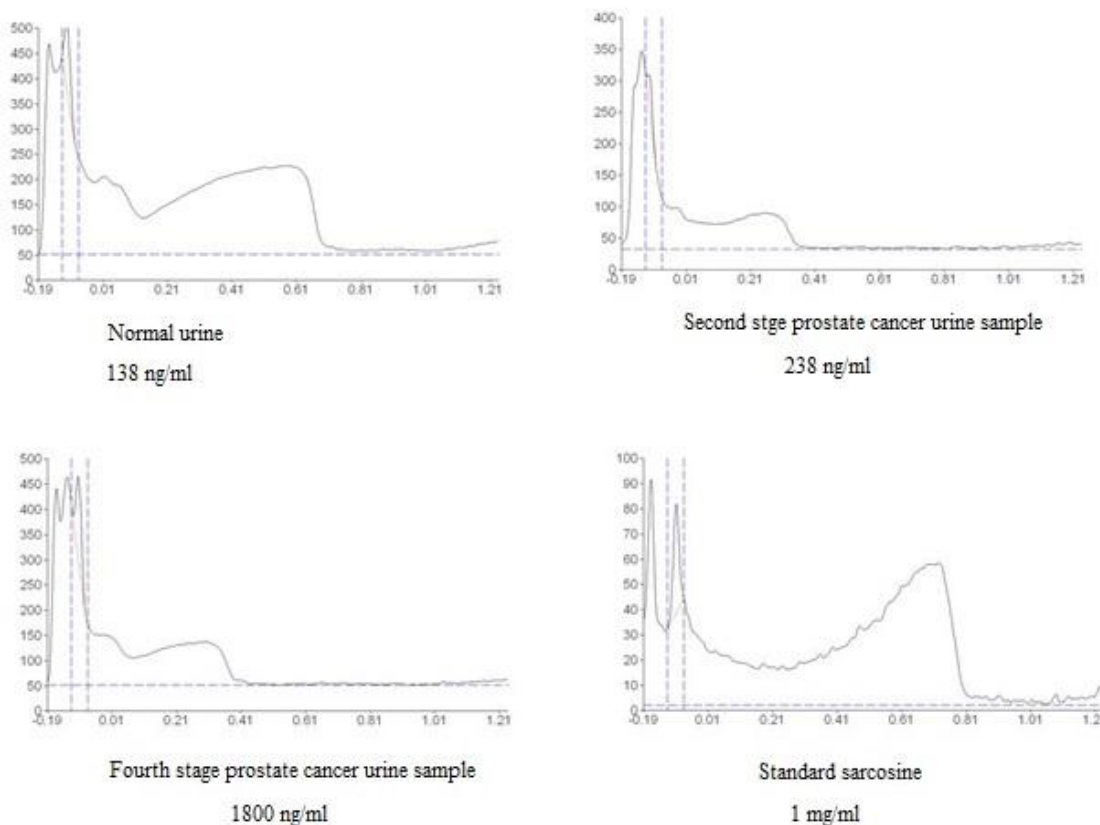
**Fig1:** Photo under ultra-violet light of a HPTLC plate. Solvent system: chloroform-acetone-toluene-methanol-diethyl amine.



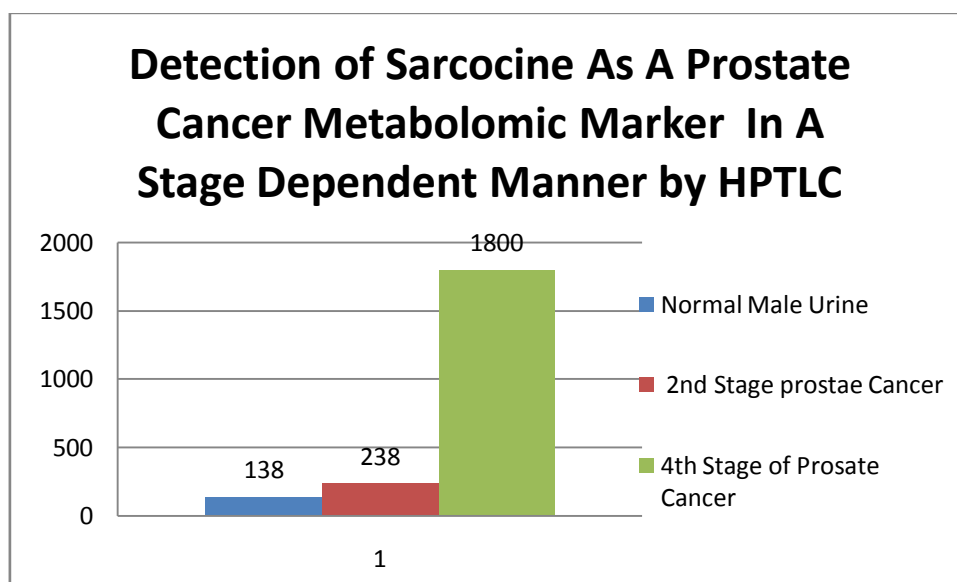
**Fig2:** HPTLC scan showing standard Sarcosine peak.

### IV. Evaluation Of Sarcosine In Prostate Cancer Patient'S Urine:

This biomarker determination technique was used for evaluation of sarcosine level in prostate cancer human subjects. The normal range, defined within the limits of two standard deviations from the mean, was 1.75-20  $\mu\text{mol}$  per 24 h, No sex related difference was found (Bellon, 1984). After analyzing several prostate cancer urine samples we found that biomarker, sarcosine level increases with progression of the cancer i.e. stage dependant. The progression of disease will be confirmed by area under the curve (AUC) of sarcosine peak when compared with the standard sarcosine peak.



**Fig3:** Comparison between healthy male urine peaks to with prostate cancer urine samples and standard which helps to understand progression of disease.



**Fig4:** Stage dependant representation of prostate cancer metabolomic marker.

### V. Conclusion:

According to the results obtained we can conclude the above developed HPTLC model succeeds in identifying the biomarker Sarcosine present in the prostate cancer patients` urine sample. Apart from this, biomarker sarcosine can be identified in very less amount i.e. 140 ng. Above HPTLC model used is reproducible, economical and less time consuming compared with highly sensitive analytical techniques such as LC-MS/GC-MS (Burton, 2012). Furthermore, such effective models would be useful for identifying progression and prevalence of cancer (Abate-Shen.C, 2000 and Ruitar.E, 1999). As disease progresses there is marked

increase in the biomarker level (Sreekumar, 2009). The correlation between sarcosine and prostate cancer progression (Stuys, 2010 and Jentzmik, 2010) helped us to develop new model which will be useful in the future to detect prostate cancer earliest possible.

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